Protocol for the ISO 16140-2:2016 validation of the D-COUNT® method for the determination of the commercial sterility in UHT treated milks and UHT plant-based drinks
Method comparison study results and Interlaboratory study results

MicroVal study number: 2023LR126

Method/Kit name: D-COUNT® Commercial Sterility Testing

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This report contains 28 pages and 58 pages of appendices.

Foreword

This report is prepared in accordance with ISO 16140-2/A1:2024 and MicroVal technical committee interpretation of ISO 16140-2 v.1.0

Company: BioMérieux SA

Expert Laboratory: Microsept

Method/Kit name: D-COUNT® Commercial Sterility Testing

Validation standard: ISO 16140-2

Reference method: Codex Code of Hygienic practice for milk and milk products (CAC/RCP 57-2004). Appendix B: Microbiocidal 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 milks and UHT plant-based drinks -

Certification organization: Lloyd's Register QA

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
g	Acceleration speed
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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Appendices

1. Introduction

This document is a method comparison study and interlaboratory study report for the validation according to the ISO 16140-2:2016 standard of the D-COUNT® method for the determination of commercial sterility in one food category: UHT treated milks and plant-based drinks.

- The alternative method:

The D-COUNT® method is based on flow cytometry technology. The method can detect all viable microorganisms present in the sample, using viability labeling and fluorescence detection.
The D-COUNT® method ensures product quality, with rapid counting, and presence/absence tests, depending on the type of product, and the microorganisms targeted.
The D-COUNT® method is a simple 4-step process for the rapid detection of microorganisms in food products. The first 2 steps outside the system are product pre-incubation and sample preparation, following dedicated protocols. Then the products are transferred into the system for analysis.
The analysis consists of cell labeling and cell counting, before displaying the results.

- The reference method:

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

- The scope of the method is:

UHT treated milks and plant based drinks up to 1 L. In this category, 3 types will be represented: UHT liquid dairy products, UHT plant-based drinks and UHT flavoured milks.

2. Method protocols

2.1. Reference method

The reference method used is the Codex Code of Hygienic Practice for Milk and Milk Products (CAC/RCP 57-2004). Appendix B Microbiocidal control measures. Section 2.2 Process management.

Based on the Council Directive 92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products, the workflow of the method is as follows:

- Pre-incubation of the UHT milk or plant-based drink at 30°C for 15 days,
- Enumeration of the microflora of the sample using the ISO 4833-1/A1:2022 method. The dairy product samples are plated onto milk Plate Count Agar (mPCA) while the plant-based samples are plated onto Plate Count Agar (PCA).
- The sterility criterion is set at ≤ 10 CFU per 0.1 mL of product.

A sample is considered positive by the reference method if the result of the count is >10 CFU/0.1 mL of product. Packages leaking during the pre-incubation period are considered positive.

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

2.2. Alternative method

2.2.1. Principle of the method

D-COUNT® combine an automatized sample labelling and a dedicated flow cytometer specifically designed for rapid microbiological testing, either qualitative (commercial sterility) or quantitative (enumeration).

A flow cytometer consists of a fluidic part which conveys sample from original container to the flow cell in a very narrow and stable laminar flow, an optical system, for fast and sensitive analysis of the interrogated volume, generally composed of a Laser for sample excitation and fluorescence measurements devices, and an electronic system for data processing which outputs the result in terms of object counts in the total volume of sample analyzed without requiring any further data interpretation.

The D-COUNT method validated hereby is a simple 3-step process for assessing commercial sterility testing after the product has been pre-incubated in its original packaging:

1. An optional manual sample preparation which can include a mechanical (centrifugation combined with a filtration) and chemical treatments allowing to remove interfering agents and to detach micro-organisms of interest from other particles.
2. A labelling protocol where samples are placed in tubes in a sample processor automate where reagents will be added for specific fluorescence labelling of micro-organisms. The D-Count® labelling is based on a universal viability marker targeting enzymatic activity of the cells as well as membrane integrity to ensure only viable microorganisms are counted.
3. Once samples are properly labelled, they are automatically injected in the flow cytometer system. Fluorescent events present in the sample are detected individually and counted in real time in a defined volume. Besides, optical fluorescence arrangement is optimized to selectively trig electronic count on labelled micro-organisms.

Samples are treated by batch of 25 or 50 samples by the automated sample preparation unit and results obtained after the pre-incubation period with around 1 hour.

2.2.2. Protocols of the method

Two protocols are available depending on the type of matrix analyzed:

- **Protocol 720:** up to 1 L UHT milks and up to 1 L UHT flavoured milks:
- pre-incubation of samples at 30°C for a period ranging from 48 to 51 hours,
- homogenization of the sample by several successive inversions and transfer of 0.5 mL of sample into a 20 mL tube,
- direct analysis using the D-COUNT system

- **Protocol 714:** up to 1 L plant-based drinks:
- pre-incubation of samples at 30°C for a period ranging from 72 to 75 hours,
- homogenization of the sample by several successive inversions and transfer of 0.5 mL of sample into a 20 mL tube,

- addition of 6 mL of ChemSol A7 reagent, then centri-filtration using a ChemFilter 25, for 8 min at 2000 g
- direct analysis of the pellet obtained using the D-COUNT system.

In this study, the minimum incubation times was tested, i.e., 48 hours for protocol 720 and 72 hours protocol 714.
The workflows of the alternative method are presented in Appendix B.

2.2.3. Restrictions

This method is not validated for the detection of molds. These are therefore excluded from the scope.

2.3. Study design

Since the reference method and the alternative method have different incubation times, 2 distinct packs were used : One for the reference method, and the other one for the alternative method. However, the matrices used for the reference method and the alternative method come from the same production batch.
The data thus generated were interpreted according to an unpaired study design.

3. Methods comparison study

3.1. Sensitivity study

3.1.1. Protocols

- **Study protocol:**

At least 30 positive samples and 30 negative samples per protocol were analyzed by the reference method and by the alternative method. Each sample was analyzed at the minimum of the incubation time of the alternative method.
As part of the study, the non-artificially contaminated samples were analyzed first in order to highlight possible natural contamination of the product.

- **Contaminations:**

Artificial contaminations were performed according to the ISO 16140-2 requirements.

As the study is a sterility test, the samples analyzed were artificially contaminated by spiking in the pack using strains:
- in stressed vegetative form (with thermal or oxidative stress)
- in sporulated form without stress.

Each original pack was disinfected, and contamination was carried out by injection of a bacterial suspension through a septum. A second septum was placed on the first to guarantee the tightness of the pack.

The stress level was evaluated by enumerating the strain on selective and non-selective agar. When working with microorganisms for which a selective agar is not available, an agar with a stress factor was used (supplementation with salt). During this study, 6 strains were affected by the use of this protocol: *Candida parapsilosis* GVNH48, *Leuconostoc citreum* ADBW69, *Micrococcus* sp. ADLA78, *Saccharomyces cerevisiae* HQHR84, *Rhodotorula mucilaginosa* FVYW49 and *Stenotrophomonas maltophila* HWV749. In order to ensure that the stress assessment is indeed due to the protocol applied and not to the agar supplemented with salt, a count of all these non-stressed strains was carried out both on TSAYE and TSAYE agar supplemented with salt. The raw results are presented at the end of the Appendix C. The differences in log CFU/ml observed do not exceed 0.2. All stresses evaluated are therefore well above 0.5 log and come from the stress protocol applied.

For microorganisms able to sporulate, spores were used for spiking. The correct application of the sporulated forms was checked before inoculation by observation under a phase contrast microscope. For the other microorganisms, strains were stressed prior contamination of the samples.

The strains used were of origin close to the matrices analyzed and were not used more than 6 times. Sample contamination levels were targeted under 3 CFU/L, without exceeding 5 CFU/L.

All details concerning artificial contaminations are given in Appendix C.

- **Pre-incubation:**

Depending on the protocol tested for the alternative method, the pre-incubation times of the samples were different:

- **Protocol 720**: pre-incubation of samples at 30°C for 48h (range from 48 to 51 hours)
- **Protocol 714**: pre-incubation of samples at 30°C for 72h (range from 72 to 75 hours)

- **Confirmations:**

Confirmation was performed only as part of the validation study by isolating 10 µL of enriched sample on PCA + Milk or PCA agar (depending on the matrix). For positive samples, the colonies observed were identified by MALDI-TOF in order to validate the concordance with the inoculation.
In the event of a discordant result observed between the D-COUNT result and the isolation of 10 µL on agar plate, an enumeration of 1 mL of the enriched sample was carried out. A sample is considered positive if the count result is >100 CFU/ 1 ml.

- **Interpretation:**

The final results were interpreted in accordance with ISO 16140-2:2016, using the acceptability limits for the unpaired methods for one category.

3.1.2. Number and nature of the samples

133 samples were tested. The distribution of positive and negative samples tested per type and per protocol is given in table 1.

Table 1: Distribution of the positive and negative samples per type and per protocol

Category	Type		Positive*	Negative	Total
UHT treated milks and plant-based drinks up to 1L	a	UHT milk	18	15	33
	c	UHT flavoured milk	18	20	38
	Total (protocol 720)		**36**	**34**	**70**
	b	UHT plant-based drinks	30	33	63
	Total (protocol 714)		**30**	**33**	**63**
TOTAL (protocol 720+714)			**66**	**67**	**133**

*Positive by at least one of the methods

3.1.3. Results

Raw data are shown in Appendix D.

When presenting the first version of the results, the MVTC asked to exclude from the statistical interpretation 4 non-artificially contaminated samples which gave a positive result only with the alternative method (n°2514966, n°2514968, n°2514971 et n°2588507). In order to have the right number of samples, 4 new artificially contaminated samples were analyzed (n°266406, n°2664047, n°2684344 and n°2684346). These four new samples gave a positive result by the two methods tested. The statistical interpretation presented below takes these modifications into account.

Table 2 shows the results of the sensitivity study for the types and the protocols tested.

Table 2: interpretation of the sensitivity study results (R+/-: reference method positive or negative, A+/-: alternative method positive or negative, PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumptive positive before confirmation)

Category	Protocol	Response	R+	R-
UHT treated milks and plant-based drinks up to 1L	**720**	A+	PA = 28	PD = 5
		A-	ND = 3 incl. 0 PPND	NA = 34 incl. 1 PPNA
	714	A+	PA = 23	PD = 2
		A-	ND = 5 incl. 0 PPND	NA = 33 incl. 0 PPNA
	Total	A+	**PA = 51**	**PD = 7**
		A-	**ND = 8 incl. 0 PPND**	**NA = 67 incl. 1 PPNA**

3.1.4. Calculation of relative trueness (RT), sensitivity (SE) and false positive ratio (FPR)

The set of results obtained were used to calculate the relative trueness, the sensitivity and the false positive ratio for each of the categories and for all the categories, according to the formulas set out in the EN ISO 16140-2/A1:2024 standard.

This interpretation is shown below in Table 3.

The calculations formulae of the ISO 16140-2 standard are presented below:

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

Table 3: values in % of sensitivity for the two methods, relative trueness and false positive ratio for the alternative method (SEalt : sensitivity for the alternative method, SEref : sensitivity for the reference method, RT: relative trueness, FPR: false positive ratio for the alternative method)

Category		Type	PA	NA	PD	ND	PPND	PPNA	SE $_{alt}$ %	SE $_{ref}$ %	RT %	FP %
UHT treated milks and plant-based drinks	a	UHT milk	15	15	2	1	0	0	94.4	88.9	90.9	0.0
	c	UHT flavoured milk	13	19	3	2	0	1	88.9	83.3	86.8	5.3
		Total (protocol 720)	**28**	**34**	**5**	**3**	**0**	**1**	**91.7**	**86.1**	**88.7**	**2.9**
	b	UHT plant-based drinks	23	33	2	5	0	0	83.3	93.3	88.9	0.0
		Total (protocol 714)	**19**	**33**	**2**	**5**	**0**	**0**	**83.3**	**93.3**	**88.9**	**0.0**
All (protocol 720+714)			**47**	**67**	**7**	**8**	**0**	**1**	**87.9**	**89.4**	**88.8**	**1.5**

The results for each protocol tested are summarized in Table 4.

Table 4: summary of the results for each protocol tested

Parameter	Formula EN ISO 16140-2 :2016	Results for protocol 720	Results for protocol 714	Results for protocol 720 + 714
Sensitivity of the alternative method (SE_{alt})	$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\ \%$	91.7 %	83.3 %	87.9 %
Sensitivity of the reference method (SE_{ref})	$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100\ \%$	86.1 %	93.3 %	89.4 %
Relative trueness (RT)	$RT = \frac{(PA + NA)}{N} \times 100\ \%$	88.7 %	88.9 %	88.8 %
False positive ratio (FPR)	$FPR = \frac{FP}{NA} \times 100\ \%$	2.9 %	0.0 %	1.5 %

3.1.5. Analysis of the discordant results

Discordant results are examined according to the standard ISO 16140-2:2016.
The positive deviations are given in table 5 and the negative deviations in table 6.

Table 5: positive deviations

Protocol	Type	#	Sample	Contamination	Strain	Inoculation level	ISO 4833-1▪		D-COUNT 720: 48 hours - 30°C / 714: 72 hours - 30°C				
							Result	Final result	Result	Confirmation	Identification	Final result	Agreement
720	a	2514921	Skimmed milk	Artificial contamination	*Escherichia coli*	1,2	<1	A	+	+ (AH)	*Escherichia coli*	P	PD
	a	2514920	Semi-skimmed organic milk	Artificial contamination	*Bacillus circulans*	4,6	<1	A	+	+ (AM)	*Bacillus circulans*	P	PD
	c	2514929	Chocolate milk	Artificial contamination	*Escherichia coli*	4	<1	A	+	+ (AH)	*Escherichia coli*	P	PD
	c	2514927	Coffe milk	Artificial contamination	*Bacillus cereus*	1,4	<1	A	+	+ (AM)	*Bacillus cereus*	P	PD
	c	2536499	Chocolate milk	Artificial contamination	*Bacillus toyonensis*	1,8	<1	A	+	+ (AM)	*Bacillus toyonensis*	P	PD
714	b	2588552	Soy and chocolate drink	Artificial contamination	*Micrococcus sp.*	0,4	<1	A	+	+ (AM)	*Micrococcus (Kocuria)*	P	PD
	b	2588545	Almond drink	Artificial contamination	*Rhodotorula mucilaginosa*	1,0	<1	A	+	+ (AH)	*Rhodotorula mucilaginosa*	P	PD

Table 6: negative deviations

Protocol	Type	#	Sample	Contamination	Strain	Inoculation level	ISO 4833-1▪		D-COUNT 720: 48 hours - 30°C / 714: 72 hours - 30°C				
							Result	Final result	Result	Confirmation	Identification	Final result	Agreement
720	a	2514918	Goat's milk semi skimmed	Artificial contamination	*Bacillus coagulans*	2.8	3.8E+06	P	-	ø	/	A	ND
	c	2536500	Milk coffee Latte classico	Artificial contamination	*Bacillus toyonensis*	1.8	1.2E+05	P	-	ø	/	A	ND
	c	2536501	Strawberry milk	Artificial contamination	*Bacillus toyonensis*	1.8	2.3E+05	P	-	ø	/	A	ND
714	b	2588547	Drink made from corn, oats, almonds	Artificial contamination	*Micrococcus sp.*	0.4	>3 10^7	P	-	ø	/	A	ND
	b	2588560	Organic drink made from chestnut, rice and oats	Artificial contamination	*Paenibacillus polymyxa*	3.4	8.7E+06	P	-	ø	/	A	ND
	b	2588561	Organic drink made from rice and vanilla	Artificial contamination	*Paenibacillus polymyxa*	3.4	2.8E+05	P	-	ø	/	A	ND
	b	2588566	Vanilla-flavored plant-based drink made from peas	Artificial contamination	*Lysinibacillus fusiformis*	1.2	2.0E+06	P	-	ø	/	A	ND
	b	2588558	Organic millet drink	Artificial contamination	*Enterobacter cloacae*	0.2	2.7E+06	P	-	ø	/	A	ND

Seven positive deviations were observed: seven from artificially contaminated samples and four from naturally contaminated samples. Among these eleven positive deviations, five were obtained with protocol 720 and six with protocol 714.
All positive results obtained by D-COUNT method were also confirmed by the additional confirmation protocol i.e. by streaking 10 µL of pre-enriched sample on plate count agar.

Eight negative deviations were observed: all from artificially contaminated samples.
The presence of microorganism in the alternative method enriched sample was neither detected by D-COUNT nor by the additional confirmation protocol by streaking 10 µL of pre-enriched sample on plate count agar. Enumerations performed on 1 ml of pre-enriched samples also gave negative results.

In conclusion, the positive deviations and the negative deviations most probably come from the nature of the study design. In an unpaired study, because of the difference of sampling between the two methods, and the use of seeded samples with low levels of contamination, no cell of microorganism may have been present in the sampling of one of the two methods.

3.1.6. Confirmations

One difference was observed between the D-COUNT result and the additional confirmation for sample n°2514914 “Vanilla milk”. D-COUNT gave a positive result, but the confirmation tests did not highlight the presence of any microorganism. Three new D-COUNT tests were carried out from the cold-stored pre-enriched sample, and they all gave a negative result.
Three additional samples of vanilla-flavored milk were analyzed by the alternative method and the reference method, and all gave negative results. These samples were not included in the statistical interpretation, but the results are available in the Appendix D.

3.1.7. Calculation and interpretation of data

Table 7 shows the difference between negative deviations and positive deviations and the acceptability limits for each protocol tested.

Table 7: acceptability limits

Protocol	Type	N+	ND	PD	Unpaired protocol	
					ND-PD	AL
720	a	18	1	2	/	/
	c	18	2	3		
	Total	**36**	**3**	**5**	**-2**	**3**
714	b	30	5	2	/	/
	Total	**30**	**5**	**2**	**3**	**3**
All protocols (720 + 714)		**66**	**8**	**7**	**1**	**3**

The observed values (ND – PD) are below the acceptability limit for each protocol tested and for all protocols combined. The alternative method produces results comparable to the reference method.

3.1.8. Conclusion of the sensitivity study

The statistical tests of the EN ISO 16140-2/A1:2024 standard conclude that the two protocols 720 and 714 of the alternative method produce results comparable to the reference method.

3.2. Relative level of detection study

3.2.1. Matrix-pairs strains

The relative level of detection was determined for each protocol tested and for combined protocols. Table 8 summarizes the matrix-strain pairs tested.

Table 8: matrix-strain pairs for the RLOD study

Category	Protocol	Test sample	Matrix	Strain	Origin	Inoculation protocol
UHT treated milks and dairy alternative drinks	**720**	1 L	UHT milk	*Stenotrophomonas maltophila* HWV749	Dairy environment	Spiking with heat stress
	714	1 L	Soy drink	*Bacillus subtilis* WJM246	Vegetal product	Spiking with spore form

3.2.2. Contamination protocol

For each matrix-strain pairs, three levels of contamination were prepared consisting of a negative control level, a low level, and a higher level.

The negative control level shall not produce positive results. Five replicates were tested for this level.
The low level shall be the theoretical detection level, it was contaminated at 0.7 - 1 CFU per pack to obtain fractional recovery results. Twenty replicates were tested for this level.
The higher level shall be just above the theoretical detection level, it was contaminated at 2 - 3 CFU per pack. Five replicates were tested for this level.

The *Stenotrophomonas maltophila* strain was stressed by a heat shock for 10 minutes at 56°C and the stress was evaluated at 1.04 log. Individual inoculation of the UHT milk packs was carried out.
For *Bacillus subtilis*, it is a sporulated form which was inoculated into the soy drink packs.

Samples were then analyzed by the reference method and the alternative method.

3.2.3. Results

The detailed results table is set out in Appendix E for the 2 protocols tested during the study.

The RLOD is defined as the ratio of the LODs of the alternative method and the reference method: RLOD= LOD_{alt} / LOD_{ref}.
The RLODs calculations were performed according to the standard ISO 16140-2: 2016 using the Excel spreadsheet available for download at http://standards.iso.org/iso/16140, with unknown concentrations. Values of the RLODs are set out in table 9.

Table 9: RLODs values for all categories (RLOD: the estimated relative level of detection value, RLODU: the upper limit of the 95% confidence interval for RLOD, RLODL: the lower limit of the 95% confidence interval for RLOD, b=ln(RLOD): logarithm of the RLOD value, sd(b): standard deviation of b, z-Test statistic: absolute value of the test statistic of the z-Test with the null hypothesis H0: b=0, p-value: p-value of the z-Test)

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value	AL
UHT milk / *Stenotrophomonas maltophila* Protocol 720	**0.868**	0.392	1.926	-0.141	0.398	0.354	1.277	**2.5**
Plant-based drink / *Bacillus subtilis* Protocol 714	**1.205**	0.566	2.565	0.186	0.378	0.492	0.623	**2.5**
Combined	**1.055**	**0.622**	**1.788**	**0.053**	**0.264**	**0.202**	**0.84**	**2.5**

3.2.4. Interpretation and conclusion

The RLODs values are below the acceptability limit set at 2.5 for “unpaired” categories described in ISO 16140-2:2016.

3.2.5. Expression of the LOD values

The LOD_{50} calculations according to Wilrich & Wilrich POD-LOD calculation program - version 12, are given in table 10.

Table 11: LOD50% for the alternative and reference method

Protocol	Reference method	Alternative method
UHT milk / *Stenotrophomonas maltophila* Protocol 720	0.531 [0.319 - 0.884]	0.470 [0.282 – 0.783]
Plant-based drink / *Bacillus subtilis* Protocol 714	0.637 [0.372 - 1.091]	0.788 [0.455 – 1.367]
Combined	0.580 [0.401 – 0.840]	0.615 [0.424 – 0.891]

3.3. Inclusivity study

3.3.1. Inclusivity study: protocol and results

- **Protocol:**

Fifty strains were cultured in brain heart broth and incubated at appropriate temperature. Dilutions were carried out in order to inoculate between 10 and 100 CFU per 1 liter pack of UHT milk. For spore-forming microorganisms, the sporulated form was inoculated and for other microorganisms, the vegetative form was inoculated. Pre-incubation of the UHT milk packs was carried out at 30°C for 48 hours and protocol 720 was applied with the D-COUNT system.

- **Results:**

All the results are presented in Appendix F.

Among the fifty strains tested, forty-eight gave a positive result with the D-COUNT protocol 720 (i.e. after 48 hours pre-incubation time).
A *Bacillus subtilis* strain (NBW594, origin: industrial water) gave a negative result with the D-COUNT and after streaking on Plate Count Agar media. This strain also gave a negative result with the reference method. Another *Bacillus subtilis* strain (WJM246, origin: curcuma) was tested and gave a positive result with the D-COUNT and Plate Count Agar media. The origin of the strain NBW594 may not be suitable for a growth in UHT milk.
A *Micrococcus luteus* strain (NBV721, origin: pond water) gave a negative result with the D-COUNT and after streaking on Plate Count Agar media. This strain was retested with the D-COUNT method after 72 hours of pre-incubation and gave a positive confirmed result. A pure culture strain was tested with the D-COUNT and gave a positive result. It is important to specify that this strain gave a negative result with the reference method. Another *Micrococcus luteus* strain (SKZ371, origin: water) was tested with the D-COUNT protocol 720 and the reference method and gave also negative results. A pure culture strain was tested with the D-COUNT and gave a positive result.
Following these growth differences between the alternative method and the reference method, the MVTC asked the Expert Laboratory to deepen the tests on the *Micrococcus luteus* strain. Ninety-two CFU of *Micrococcus luteus* NBV721 were inoculated per 1 liter pack of UHT milk. Alternative method and enumeration with mPCA were applied at different incubation times: 24h, 48h, 5 days, 6 days, 8 days, 9 days, 13 days, 14 days and 15 days. The raw results are presented in Appendix F. These additional tests showed that the *Micrococcus luteus* strain is well detected by the D-COUNT method but after an incubation time of 8 days. The counts carried out highlight very slow growth of this strain in the milk matrix (only 45 CFU/ml after 6 days of incubation).
These new tests allowed the detection of the strain with the reference method.

For two other strains (ESKW37: *Streptococcus thermophilus* and HPDM67: *Levilactobacillus brevis*), a positive result was observed with the D-COUNT system, but no growth was observed after streaking on Plate Count Agar. For the *Streptococcus thermophilus* strain, a confirmation was possible only after streaking enriched UHT milk on Plate Count Agar media or MRS media incubated at 44°C. For the *Levilactobacillus brevis* strain, a confirmation was possible only after streaking enriched UHT milk on M17 agar media incubated at 30°C.

3.3.2. Conclusion inclusivity study

The inclusivity of the alternative method is satisfactory.

3.4. Conclusion of the method comparison study

The method comparison study of the method was performed according to the EN ISO 16140-2/A1:2024 standard. The acceptance criteria are fulfilled by the D-COUNT® Commercial Sterility Testing method.

- **Sensitivity study**

During this method comparison study, the performance of the D-COUNT® method was compared to that of the Codex Code of Hygienic Practice for Milk and Milk Products (CAC/RCP 57-2004). (Annex B Microbiocidal control measures. Section 2.2 Process management) method by analyzing 133 samples divided into 2 protocols.
The observed values (ND – PD) were below the acceptability limits for each protocol and for all protocols.
Statistically, the two protocols proposed for the 2 matrices type tested for alternative method produces results comparable to that of the reference method.

- **Relative level of detection study**

The relative detection level of the D-COUNT® method and reference method was evaluated by artificially contaminating matrices of the application scope.
The D-COUNT™ method and the reference method showed similar LODs values for the two protocols tested.
The RLODs values are below the acceptability limit set at 2.5 for “unpaired” category described in ISO 16140-2:2016.

- **Inclusivity study**

The specificity of the method is satisfactory, as all target strains were detected (inclusivity).

4. Interlaboratory study

4.1. Interlaboratory study organization

Samples were sent to 8 different laboratories located in 3 different European countries. For some of these laboratories, 2 collaborators participated in the study. During the interlaboratory study, the protocol 720 was applied.

- **Strain and matrix**

This study was done with UHT milk inoculated with a *Microbacterium arborescens* strain, coded JEGZ55, isolated from a dairy product.
To facilitate the study, 250 mL UHT milk bottles were used. They were all purchased from a supermarket, and all belong to the same production batch.

- **Labelling and shipping**

Samples were individually inoculated on Monday, September 23, 2024; as described below:

- 24 blind coded samples (red dot) for the determination of commercial sterility by the alternative method,
- 24 blind coded samples (blue dot) for the determination of commercial sterility by the reference method,
- 1 water flask labelled “Temperature Control” with a temperature probe for temperature control during transport and storage in the laboratory until the beginning of the analysis.

The targeted inoculation levels in the UHT milk bottle were the following:

- Level 0: 0 CFU/pack,
- Level 1: 1 CFU/pack,
- Level 2: 5 CFU/pack.

At least one of these inoculated levels should produce fractional positive results.

Each laboratory received 48 samples, i.e. 8 samples per inoculation level and method.
Blind coded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different collaborators.
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 analysis.

Samples were shipped within 24 h to 48 h to each of the participating laboratories. The temperature conditions had to stay lower or equal to 8°C during transport, and between 0°C – 8°C on arrival at each of the collaborators. After receipt in the various laboratories, the samples were stored in the cold until Wednesday September 25, the start date of analysis for all laboratories.

Collaborative study laboratories and the expert laboratory carried out the analysis on Day 2 (i.e. September 25) with the alternative and reference methods.

The collaborative study instructions were sent on Wednesday, September 11, 2024.

4.1.1. Experimental parameters controls

4.1.1.1. Commercial sterility of the matrix before inoculation

The sterility of the packs was checked on 6 random samples, analyzed with the reference method. All results obtained were negative.

4.1.1.2. Strain stability

In order to evaluate the stability of the *Microbacterium arborescens* strain during transport, counts were carried out at different times, between inoculation and after 4 days of cold storage. The results are presented in Table 12.

Table 12: stability of the strain

Stability of the strain							
Day	**Test 1**		**Test 2**		**Test 3**		***m* tests**
	CFU/ml	**log CFU/ml**	**CFU/ml**	**log CFU/ml**	**CFU/ml**	**log CFU/ml**	**log CFU/ml**
Day 0	970	*2,99*	920	*2,96*	850	*2,93*	*2,96*
Day 2	990	*3,00*	950	*2,98*	910	*2,96*	*2,98*
Day 3	1000	*3,00*	1100	*3,04*	1000	*3,00*	*3,01*
Day 4	1800	*3,26*	1700	*3,23*	1200	*3,08*	*3,19*

The results show very good stability of the strain even after 4 days of storage.

4.1.1.3. Contamination levels

The samples were inoculated by a *Microbacterium arborescens* strain. A culture was prepared in a Brain Heart Infusion broth incubated overnight at 30°C. The purity of the strain was checked on non-selective agar. Successive decimal dilutions were carried out in order to reach the targeted contamination levels for levels L_1 and L_2. These levels were checked before inoculation by counting on plates of PCA + milk the day preceding inoculation. On the day of inoculation of the packs, the real level of each level was measured with ten enumerations.
The results are presented in Table 13.

Table 13: Contamination levels and sample codifications

Level	Samples	Theoretical target level (CFU/250 mL)	True level (CFU/250 mL)
Level 0 (L_0)	2-5-9-12-14-16-19-23 26-29-33-36-38-40-43-47	0	**/**
Low level (L_1)	1-4-7-10-18-20-21-24 25-28-31-34-42-44-45-48	1	0/0/0/2/2/2/2/1/2/1 = **1,2**
High level (L_2)	3-6-8-11-13-15-17-22 27-30-32-35-37-39-41-46	5	6/2/3/3/5/1/6/7/7/7 = **4,7**

4.1.1.4. Logistic conditions

The temperatures measured upon receipt by the labs and those registered by the thermo-probes, along with the receipt dates are given in Table 14.

All the samples were delivered in appropriate conditions. For most laboratories, the temperature measured upon receipt by each of them is between 4,2°C and 8,1°C. Note, for Laboratory H, the temperature measured upon receipt is 12,1°C (10,1°C measured by the recorder).

Table 14: Sample temperatures at receipt

Lab	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	State of the samples	Analysis date
A	2,4	5,2	24/09/24 - 14h00	Good	25/09/24
B	2,6	6,4	24/09/24 - 14h00	Good	25/09/24
C	8.2	7,7	24/09/24 - 09h10	Good	25/09/24
D	6,2	5,7	24/09/24 - 12h00	Good	25/09/24
E	6,0	5,6	24/09/24 - 12h00	Good	25/09/24
F	3,9	6,2	24/09/24 - 16h00	Good	25/09/24
G	4,2	6,1	24/09/24 - 17h45	Good	25/09/24
H	10,1	12,1	25/09/24 - 12h00	Good	25/09/24
I	8,9	8,1	25/09/24 - 12h00	Good	25/09/24
J	*Not measured*		25/09/24 - 11h30	Good	*Not analysed*
K	9,1	3,4	25/09/24 - 11h30	Good	25/09/24
L	2,6	4,2	25/09/24 - 11h30	Good	25/09/24
M	7,2	7,1	25/09/24 - 10h00	Good	25/09/24
N	4.7	6,9	25/09/24 - 10h00	Good	25/09/24

All collaborators received their packages within 48 hours maximum. No damaged packages have been reported.
All laboratories analyzed the samples on Wednesday September 25 except for laboratory J which ran out of time.

4.2. Interlaboratory study results

4.2.1. Expert laboratory results

The results obtained by the expert lab are given in Table 15. Raw results are in Appendix G.

Table 15: Results obtained by the expert Lab.

Level	Reference method	Alternative method
L_0	0/8	0/8
L_1	5/8	6/8
L_2	8/8	8/8

4.2.2. Results obtained by the collaborators

14 collaborators participated to the study. The results obtained are provided for the 2 methods in Table 16. Raw results are in Appendix H.

Table 16: Positive results by the reference and alternative methods

Lab	Reference method			D-COUNT method		
	L_0	L_1	L_2	L_0	L_1	L_2
A	1/8	7/8	8/8	0/8	6/8	8/8
B	1/8	5/8	8/8	0/8	5/8	8/8
C	2/8	4/8	8/8	0/8	**0**/8	**1**/8
D	0/8	7/8	8/8	0/8	7/8	8/8
E	0/8	8/8	8/8	0/8	8/8	8/8
F	2/8	6/8	8/8	0/8	5/8	8/8
G	0/8	5/8	8/8	0/8	7/8	8/8
H	0/8	7/8	8/8	0/8	4/8	8/8
I	0/8	4/8	8/8	0/8	5/8	8/8
J	*Not analyzed*					
K	0/8	5/8	8/8	0/8	6/8	8/8
L	3/8	4/8	8/8	0/8	4/8	8/8
M	0/8	7/8	7/8	0/8	5/8	8/8
N	0/8	7/8	8/8	0/8	5/8	8/8
TOTAL	**9/104**	**76/104**	**103/104**	**0/104**	**67/104**	**97/104**

An issue was reported by two Collaborators:
- Laboratory J did not have time to carry out the requested analyses,
- Laboratory C reported that the incubator used to incubate the samples from the alternative method never reached the temperature of 30°C required by the alternative method. Upon receipt, the samples were placed directly in the incubator and the temperature controlled by the probe never reached 30°C. (see curve below in Figure 1)

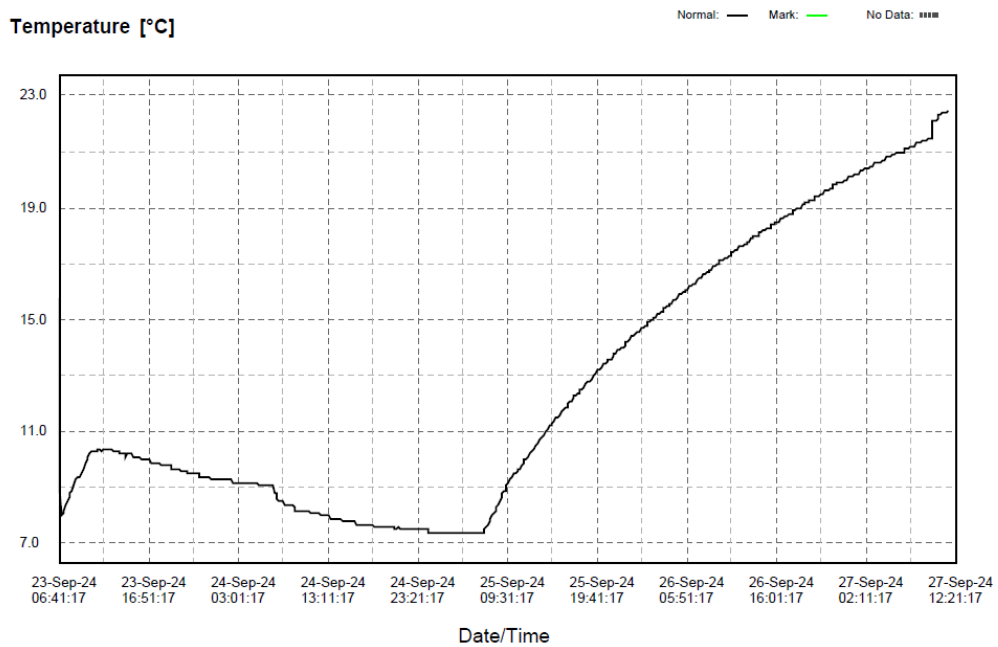


Figure 1: Laboratory C incubation conditions

Laboratories C and J are therefore excluded of the study.

Some positive results were obtained at level L_0 (i.e. on non-inoculated samples) with the reference method only. These are the following laboratories:

- Laboratory A: 1 sample
- Laboratory B: 1 sample
- Laboratory F: 2 samples
- Laboratory L: 3 samples

A root cause analysis was carried out by the Expert laboratory to explain these results.

- **For Laboratory A**, this relates to sample n°26. The results obtained are as follows:
  Pure: >100 UFC/mL
  10^{-1}: >100 UFC/mL
  10^{-2}: <100 UFC/mL
  10^{-3}: <100 UFC/mL
  The plates not having been preserved, the Expert Laboratory asked to retest sample n°26. All dilutions carried out gave a negative result.

- **For Laboratory B,** this relates to sample n°47. The results obtained are as follows:
  Pure: >100 UFC/mL
  10^{-1}: >100 UFC/mL
  10^{-2}: >100 UFC/mL
  10^{-3}: >100 UFC/mL
  As collaborator A and B were in the same Laboratory, the plates were not kept, the Expert Laboratory asked to reanalyze sample n°47. All dilutions carried out gave a negative result.

- **For Laboratory F**, this relates to samples n°33 and n°36. The results obtained are as follows:
  Sample n° 33 Pure: 0 UFC/mL
  10^{-1}: 0 UFC/mL
  10^{-2}: 64 UFC/mL
  10^{-3}: 49 UFC/mL

  Sample n° 36 Pure: 76 UFC/mL
  10^{-1}: 41 UFC/mL
  10^{-2}: 30 UFC/mL
  10^{-3}: 9 UFC/mL

  No agreement between the successive dilutions was observed for these 2 samples. Retests were carried out and gave negative results for sample n°36 and 1 colony for pure plate and 1 colony for plate 10^{-3} for sample n°33. This highlights a cross-contamination during the initial analysis

- **For Laboratory L,** this relates to samples n°26, n°29 and n°33. The results obtained are as follows:
  Sample n° 26 Pure: 1 UFC/mL

10^{-1}: 0 UFC/mL
10^{-2}: >300 UFC/mL
10^{-3}: 1 UFC/mL

Sample n° 29 Pure: 34 UFC/mL
10^{-1}: 0 UFC/mL
10^{-2}: 0 UFC/mL
10^{-3}: >300 UFC/mL

Sample n° 33 Pure: 0 UFC/mL
10^{-1}: 37 UFC/mL
10^{-2}: 0 UFC/mL
10^{-3}: 7 UFC/mL

No agreement between the successive dilutions was observed for these 3 samples. For this collaborator, the Expert Laboratory managed to recover the 3 samples as well as the Petri dishes showing colonies. Retests were carried out by the Expert Laboratory on the 3 samples, and all gave negative results.
Moreover, the observation of the Petri dishes presenting colonies showed that they are very probably due to cross-contamination. The different types of colonies observed were purified and identified by RNA 16S amplification and Sanger sequencing. The report is available in Appendix E. The results obtained are as follows:

RESULTATS DES ANALYSES Blast

SOUCHE	*RESULTAT IDENTIFICATION*	*% d'identité*	*score*
	BACTERIES		
VDM-L33-10^{-3}	Streptococcus_salivarius	99.87%	2724
VDM-L33-10^{-1}	Staphylococcus_epidermidis	99.93%	2713
VDM-L29-10^{-3}	Streptococcus_salivarius	99.87%	2724
VDM-L26-10^{-2}	Staphylococcus_epidermidis	99.93%	2713
VDM-L26-PUR	Staphylococcus_epidermidis	99.93%	2713

Figure 2: Laboratory L: identification of colonies observed

For the 3 contaminated samples (L26, L29 and L33), the identifications highlighted the presence of commensal bacteria of the human skin and oral sphere. The strain of *Microbacterium arborescens*, inoculated in the milk flasks, has never been found. A cross-contamination of these 3 samples most probably occurred for Laboratory F.

The root cause analysis clearly highlights that cross-contaminations occurred for Collaborators A, B, F and L. That's why the Expert laboratory proposes that all the samples taken into account by the root cause analysis are considered as negative results.

4.2.3. Results of the collaborators retained for interpretation

The results obtained with the 12 labs kept for interpretation are presented in Table 17.

Table 17: Positive results (Without Lab C and J)

Lab	Reference method			D-COUNT method		
	L_0	L_1	L_2	L_0	L_1	L_2
A	0/8	7/8	8/8	0/8	6/8	8/8
B	0/8	5/8	8/8	0/8	5/8	8/8
D	0/8	7/8	8/8	0/8	7/8	8/8
E	0/8	8/8	8/8	0/8	8/8	8/8
F	0/8	6/8	8/8	0/8	5/8	8/8
G	0/8	5/8	8/8	0/8	7/8	8/8
H	0/8	7/8	8/8	0/8	4/8	8/8
I	0/8	4/8	8/8	0/8	5/8	8/8
K	0/8	5/8	8/8	0/8	6/8	8/8
L	0/8	4/8	8/8	0/8	4/8	8/8
M	0/8	7/8	7/8	0/8	5/8	8/8
N	0/8	7/8	8/8	0/8	5/8	8/8
TOTAL	**0/96**	**72/96**	**95/96**	**0/96**	**67/96**	**96/96**

4.3. Calculation and interpretation

4.3.1. 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 L_0 are the following (See Table 18).

Table 18: Percentage of specificity

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

N: number of all L_0 tests

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

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

4.3.2. Calculation of the sensitivity (SEalt), the sensitivity for the reference method (SEref), the relative trueness (RT) and the false positive ratio for the alternative method (FPR)

Fractional positive results were obtained for the low inoculation level (L_1) and the high inoculation level (L_2).

The interpretation was thus done for these two inoculation levels.

A summary of the results of the collaborators retained for interpretation and obtained with the reference and the alternative methods for Level 1 and Level 2 is provided in Table 19.

Table 19: summary of the obtained results with the reference method and the alternative method for Level 1 and Level 2

	Response	Reference method positive (R+)	Reference method negative (R-)
L₁	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 52**	Positive deviation (R-/A+) **PD = 15**
	Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 20**	Negative agreement (A-/R-) **NA = 9**
L₂	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 95**	Positive deviation (R-/A+) **PD = 1**
	Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 0**

Based on the data summarized in Table 19, 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 20).

Table 20: sensitivity, relative trueness and false positive ratio percentages

Parameter		***L₁***	***L₂***
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)}\ x\ 100\% =$	**77,0 %**	**100 %**
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)}\ x\ 100\% =$	**82,7 %**	**98,9 %**
Relative trueness	$RT = \frac{(PA+NA)}{N}\ x\ 100\% =$	**55,2 %**	**98,9 %**
False positive ratio for the alternative method	$FPR = \frac{FP}{NA}\ x\ 100\% =$	**0,0 %**	**0,0 %**

4.3.3. Interpretation of data

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 acceptability limit (AL). The AL is defined as $[(ND - PD)_{max}]$ and calculated per level where fractional recovery was obtained as described below using the following three parameters:

- $(p+)_{ref} = \frac{P_x}{N_x}$, where

Px = number of samples with a positive result obtained with the reference method at level x, (L_1 or L_2) for all laboratories;
Nx = number of samples tested at level x (L_1 or L_2) with the reference method by all laboratories.

- $(p+)_{alt} = \frac{CP_x}{N_x}$, where

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

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

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

Nx = the total number of samples tested for level x (L1 or L2) by all laboratories.

When the observed value exceeds the AL (as described in EN ISO 16140-2/A1:2024), investigations should be made (e.g. root cause analysis) in order to provide an explanation of the observed results.

Based on the AL and the additional information, it is decided whether the alternative method is regarded as not fit for purpose. The reasons for acceptance of the alternative method in case the AL is not met shall be stated in the study report.

In this study, fractional positive results are observed at level L_1 and level L_2. The different parameters obtained by the calculation are detailed in Table 21.

Table 21: values obtained for the determination of the acceptability limit

Parameter	Level 1	Level 2
	Value	Value
$(p+)_{ref}$	0.75	0.99
$(p+)_{alt}$	0.70	1.00
Acceptability limit: AL = $(ND-PD)_{max}$	**10.7**	**1.7**
Observed value: ND-PD	**5**	**-1**

The EN ISO 16140-2/A1:2024 requirements are fulfilled as (ND - PD) meet the AL (calculated values ≤ AL) for each inoculation level.

4.3.4. Evaluation of the RLOD between laboratories

The RLOD was calculated using the EN ISO 16140-2/A1:2024 Excel spreadsheet available at http://standards.iso.org/iso/16140/-2/ed-1/en/RLOD_inter-lab-study_16140-2_AnnexF_ver1_28-06-2017.xls.

The results are used only for information (see Table 22).

Table 22: RLOD - laboratory comparison

Method	LOD50%	LOD95%	RLOD
Reference	0,53 [0,50 ; 0,79]	2,70 [2,15 ; 3,40]	**1,05 [0,81 - 1,37]**
Alternative	0,66 [0,52 ; 0,83]	2,84 [2,26 ; 3,57]	

4.4. Conclusion

The data shows no significant differences between the reference method and the alternative method. The ND-PD value meets the Acceptability Limit criteria of ND-PD_{max} L_1 and L_2 levels.
The data and interpretations comply with the EN ISO 16140-2/A1:2024 requirements.

5. General conclusion

The method comparison study conclusions are:

- **Sensitivity study**

The observed values (ND – PD) were below the acceptability limits for each protocol and for all protocols.
Statistically, the two protocols proposed for the 2 matrices type tested for alternative method produces results comparable to that of the reference method.

- **Relative level of detection study**

The relative detection level of the D-COUNT® method and reference method was evaluated by artificially contaminating matrices of the application scope.
The D-COUNT™ method and the reference method showed similar LODs values for the two protocols tested.
The RLODs values are below the acceptability limit set at 2.5 for “unpaired” category described in ISO 16140-2:2016.

- **Inclusivity study**

The specificity of the method is satisfactory, as all target strains were detected (inclusivity).

The interlaboratory study conclusions are:

The data shows no significant differences between the reference method and the alternative method. The ND-PD value meets the Acceptability Limit criteria of ND-PD_{max} L_1 and L_2 levels.
The data and interpretations comply with the EN ISO 16140-2/A1:2024 requirements.

Le Lion d’Angers, November 18, 2024

Guillaume Mesnard
Technical deputy manager

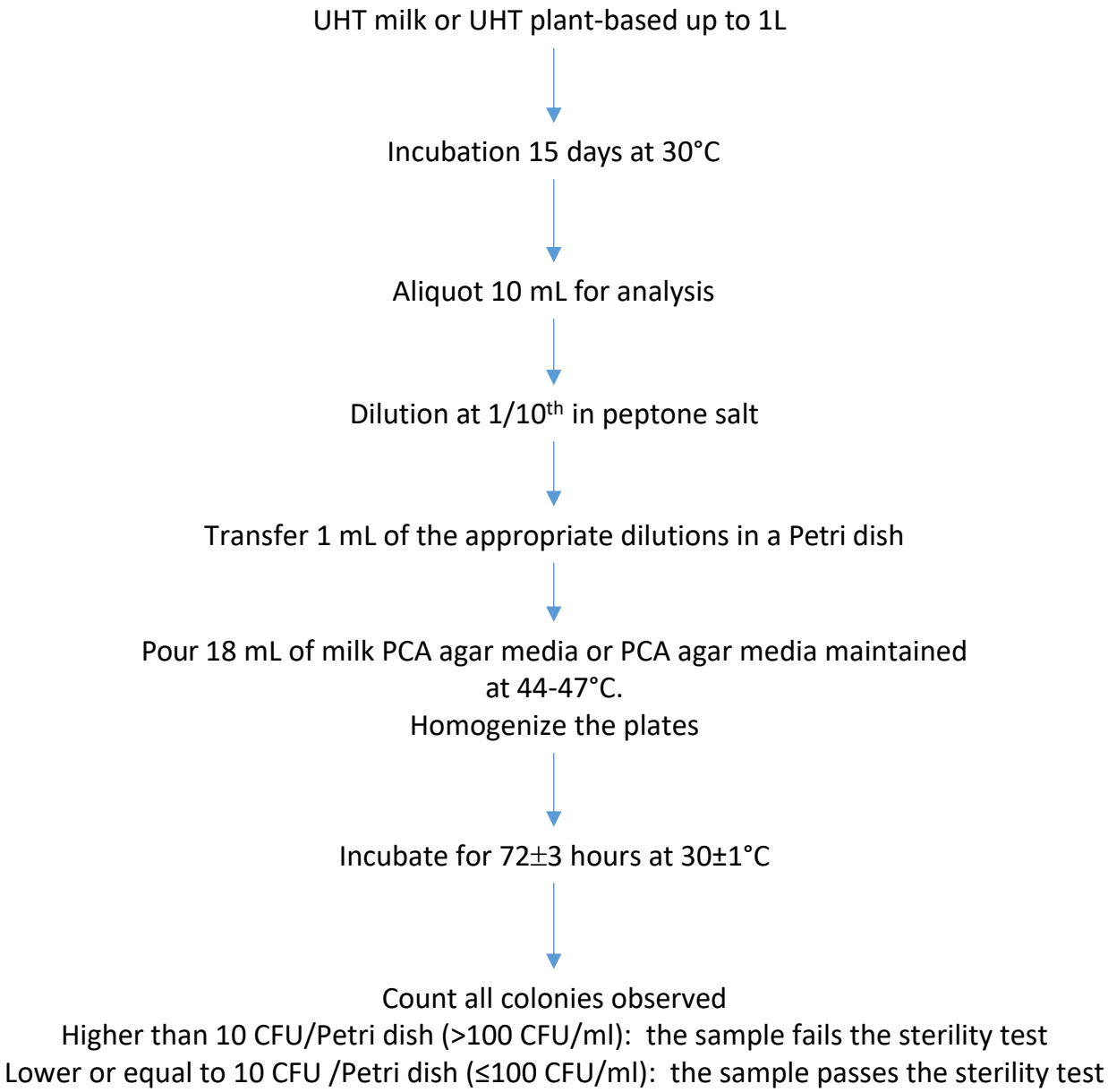
François Le Nestour
Head of the Microbiology Department

APPENDICES

APPENDIX A

PROTOCOL OF THE REFERENCE METHOD

European Directive 92/46 annex C. Chapter 2 for UHT milk,
using ISO 4833-1:2013 for the plate count at 30°C



APPENDIX B

PROTOCOLS OF THE ALTERNATIVE METHOD

D-COUNT® commercial sterility testing

Protocol 720

UHT milk or UHT flavoured milk

↓

Pre-incubation 48 hours at 30°C

↓

Aliquot 500 µL in 20 mL tube

↓

Load the 20 ml tubes on the D-COUNT
Run the 720 application

↓

Read the results

Protocol 714

UHT plant-based drinks

↓

Pre-incubation 72 hours at 30°C

↓

Aliquot 500 µL in 20 mL tube

↓

Add 6 mL of ChemSol A7 reagent and mix

↓

Centri-filtration using a ChemFilter 25
for 8 minutes at 2000 g

↓

Discard the supernatant

↓

Load the 20 ml tubes on the D-COUNT
Run the 714 application

↓

Read the results

Appendix C
Artificial contaminations

Appendix C - Artificial contaminations

Type	#	Sample	Strain	Code strain	Origin	Stress	Stress level	Inoculation level
a	2514883	Organic whole milk	*Bacillus circulans*	CQND74	Infant powder formula	Spores	/	3,2
a	2514884	Semi- skimmed milk 10 vitamins	*Enterococcus faecalis*	GCQ471	Raw milk cheese	15 minutes at 60°C	1,55	1,2
a	2514885	Semi- skimmed milk lactose free	*Listeria innocua*	BLV059 LIS SPP	Pasteurized milk cheese	30 minutes at 60°C	0,7	1,2
a	2514886	Skimmed milk	*Listeria monocytogenes*	12MOBO96LM	Raw sheep's milk	15 minutes at 56°C	2,37	3,6
a	2514887	Semi-skimmed sheep's milk	*Listeria monocytogenes*	12MOBO96LM	Raw sheep's milk	30 minutes at 55°C then 40 minutes at -20°C	0,95	0,4
a	2514921	Skimmed milk	*Escherichia coli*	TDW583	Raw milk cheese	8 minutes at 56°C	1,09	1,2
a	2514922	Semi-organic skimmed milk lactose-free	*Staphycoccus aureus*	EXX511	Raw milk cheese	15 minutes at 56°C	0,98	5,0
a	2514923	Semi-skimmed milk	*Staphycoccus aureus*	EXX511	Raw milk cheese	10 minutes at 56°C	0,8	1,8
a	2514924	Semi-organic skimmed milk	*Escherichia coli*	TDW583	Raw milk cheese	7 minutes at 56°C	0,84	1,2
a	2514919	Semi-skimmed milk	*Bacillus cereus*	FXM250	Cheese	Spores	/	0,8
a	2514920	Semi-skimmed organic milk	*Bacillus circulans*	CQND74	Infant powder formula	Spores	/	4,6
a	2514917	Semi-skimmed milk	*Paenibacillus polymyxa*	FQJ653	Environment	Spores	/	2,4
a	2514918	Goat's milk semi skimmed	*Bacillus coagulans*	FNX731	Evapored milk	Spores	/	2,8
a	2536424	Whole organic milk	*Candida parapsilosis*	GVNH48	Dairy environment	Oxidative stress H_2O_2 0,5% during 7 minutes	0,5	2,6
a	2536425	Whole organic milk	*Candida parapsilosis*	GVNH48	Dairy environment	Oxidative stress H_2O_2 0,5% during 7 minutes	0,5	4,2
a	2536426	Semi-skimmed milk	*Leuconostoc citreum*	ADBW69	Composite food	Oxidative stress H_2O_2 0,5% during 7 minutes	0,5	1,2
a	2536427	Whole organic milk	*Klebsiella pneumoniae*	CEC296	Milk	Oxidative stress H_2O_2 0,5% during 7 minutes	2,1	1,8
a	2536428	Semi-skimmed sheep's milk	*Klebsiella pneumoniae*	CEC296	Milk	Oxidative stress H_2O_2 0,5% during 7 minutes	2,1	3,6
b	2588543	Oat drink	*Bacillus pumilus*	DLX565	Minth	Spores	/	2,2
b	2588547	Drink made from corn, oats, almonds	*Micrococcus sp.*	ADLA78	Vegetal extract	10 minutes at 60°C	1,18	0,4

Appendix C - Artificial contaminations

Type	#	Sample	Strain	Code strain	Origin	Stress	Stress level	Inoculation level
b	2588548	Rice drink	*Bacillus pumilus*	DLX565	Minth	Spores	/	2,2
b	2588552	Soy and chocolate drink	*Micrococcus sp.*	ADLA78	Vegetal extract	10 minutes at 60°C	1,18	0,4
b	2588553	Soy drink with sugar	*Bacillus pumilus*	DLX565	Minth	Spores	/	2,2
b	2588557	Organic cashew drink	*Micrococcus sp.*	ADLA78	Vegetal extract	10 minutes at 60°C	1,18	0,4
b	2588560	Organic drink made from chestnut, rice and oats	*Paenibacillus polymyxa*	FQJ653	Environment	Spores	/	3,4
b	2588561	Organic drink made from rice and vanilla	*Paenibacillus polymyxa*	FQJ653	Environment	Spores	/	3,4
b	2588562	Organic spelled drink	*Paenibacillus polymyxa*	FQJ653	Environment	Spores	/	3,4
b	2588563	Organic protein drink made from yellow peas	*Paenibacillus polymyxa*	FQJ653	Environment	Spores	/	3,4
b	2588564	Organic almond cappuccino drink	*Lysinibacillus fusiformis*	BMH115	Dairy product with fruits	Spores	/	1,2
b	2588565	Organic almond pea protein drink	*Lysinibacillus fusiformis*	BMH115	Dairy product with fruits	Spores	/	1,2
b	2588566	Vanilla-flavored plant-based drink made from peas	*Lysinibacillus fusiformis*	BMH115	Dairy product with fruits	Spores	/	1,2
b	2588567	Sugar-free plant-based drink made from peas	*Saccharomyces cerevisiae*	HQHR84	Dairy product	Oxidative stress H_2O_2 0,5% during 3 minutes	0,51	3,0
b	2588540	Oat drink enriched with calcium and vitamin	*Rhodotorula mucilaginosa*	FVYW49	Leaf artichoke	Oxidative stress H_2O_2 0,5% during 10 minutes	0,52	1,0
b	2588541	Vanilla flavored almond drink	*Enterobacter cloacae*	DELF25	Cinnamon	10 minutes at 60°C	0,50	0,2
b	2588542	Roasted almond milk drink without sugar	*Pantoea agglomerans*	HSD757	Oats	Oxidative stress H_2O_2 0,5% during 10 minutes	0,60	1,8
b	2588544	Hazelnut drink	*Bacillus cereus pseudomycoïdes*	FFT320	Vegetal extract	Spores	/	1,0
b	2588545	Almond drink	*Rhodotorula mucilaginosa*	FVYW49	Leaf artichoke	Oxidative stress H_2O_2 0,5% during 10 minutes	0,52	1,0
b	2588546	Pea protein drink	*Pantoea agglomerans*	HSD757	Oats	Oxidative stress H_2O_2 0,5% during 10 minutes	0,60	1,8
b	2588549	Macchiato flavored soy drink	*Bacillus cereus pseudomycoïdes*	FFT320	Vegetal extract	Spores	/	1,0
b	2588550	Chocolate flavored hazelnut drink	*Pantoea agglomerans*	HSD757	Oats	Oxidative stress H_2O_2 0,5% during 10 minutes	0,60	1,8

Appendix C - Artificial contaminations

Type	#	Sample	Strain	Code strain	Origin	Stress	Stress level	Inoculation level
b	2588551	Coconut drink	*Rhodotorula mucilaginosa*	FVYW49	Leaf artichoke	Oxidative stress H_2O_2 0,5% during 10 minutes	0,52	1,0
b	2588554	Plain soy drink without sugar	*Bacillus cereus pseudomycoïdes*	FFT320	Vegetal extract	Spores	/	1,0
b	2588555	Organic hemp drink	*Enterobacter cloacae*	DELF25	Cinnamon	10 minutes at 60°C	0,50	0,2
b	2588556	Organic drink with oats and Lithothamnium algae	*Rhodotorula mucilaginosa*	FVYW49	Leaf artichoke	Oxidative stress H_2O_2 0,5% during 10 minutes	0,52	1,0
b	2588558	Organic millet drink	*Enterobacter cloacae*	DELF25	Cinnamon	10 minutes at 60°C	0,50	0,2
b	2588559	Organic drink made from cereals and nuts	*Bacillus cereus pseudomycoïdes*	FFT320	Vegetal extract	Spores	/	1,0
b	2612499	Hazelnut drink	*Enterobacter cloacae*	DELF25	Cinnamon	10 minutes at 60°C	0,50	0,2
b	2615000	Spelled drink	*Pantoea agglomerans*	HSD757	Oats	Oxidative stress H_2O_2 0,5% during 10 minutes	0,60	1,8
b	2612501	Oat drink	*E. coli*	TZP821	Vegetal product	7 minutes at 56°C	1,1	0,6
b	2612673	Coconut drink with vitamin B12	*Staphylococcus aureus*	KER837	Vegetal product	15 minutes at 56°C	0,51	2,3
c	2514889	Chocolate milk	*Bacillus circulans*	CQND74	Infant powder formula	Spores	/	1,3
c	2514891	Vanilla milk	*Enterococcus faecalis*	GCQ471	Raw milk cheese	15 minutes at 60°C	1,55	3,2
c	2514892	Chocolate-caramel milk	*Listeria innocua*	BLV059 LIS SPP	Pasteurized milk cheese	30 minutes at 60°C	0,70	1,6
c	2514893	Strawberry milk	*Listeria monocytogenes*	12MOBO96LM	Raw sheep's milk	30 minutes at 55°C then 40 minutes at -20°C	0,95	0,6
c	2514894	Chocolate milk	*Listeria monocytogenes*	12MOBO96LM	Raw sheep's milk	15 minutes at 56°C	2,37	0,4
c	2514929	Chocolate milk	*Escherichia coli*	TDW583	Raw milk cheese	8 minutes at 56°C	1,09	4,0
c	2514930	Cappuccino milk	*Staphycoccus aureus*	EXX511	Raw milk cheese	15 minutes at 56°C	0,98	1,4
c	2514931	Organic chocolate milk	*Staphycoccus aureus*	EXX511	Raw milk cheese	10 minutes at 56°C	0,8	3
c	2514932	Chocolate milk	*Escherichia coli*	TDW583	Raw milk cheese	7 minutes at 56°C	0,84	3,2
c	2514927	Coffe milk	*Bacillus cereus*	FXM250	Cheese	Spores	/	1,4

Appendix C - Artificial contaminations

Type	#	Sample	Strain	Code strain	Origin	Stress	Stress level	Inoculation level
c	2514928	Vanilla milk	*Bacillus circulans*	CQND74	Infant powder formula	Spores	/	2,8
c	2514925	Hazelnut chocolate milk	*Paenibacillus polymyxa*	FQJ653	Environment	Spores	/	1,4
c	2514926	Strawberry milk	*Bacillus coagulans*	FNX731	Evapored milk	Spores	/	1,6
c	2536417	Chocolate milk	*Candida parapsilosis*	GVNH48	Dairy environment	Oxidative stress H_2O_2 0,5% during 7 minutes	0,5	2,6
c	2536418	Milk with almond	*Candida parapsilosis*	GVNH48	Dairy environment	Oxidative stress H_2O_2 0,5% during 7 minutes	0,5	4,2
c	2536419	Coffee with milk	*Leuconostoc citreum*	ADBW69	Composite food	Oxidative stress H_2O_2 0,5% during 7 minutes	0,5	1,2
c	2536420	Strawberry milk	*Klebsiella pneumoniae*	CEC296	Milk	Oxidative stress H_2O_2 0,5% during 7 minutes	2,1	1,8
c	2536421	Chocolate milk	*Klebsiella pneumoniae*	CEC296	Milk	Oxidative stress H_2O_2 0,5% during 7 minutes	2,1	3,6
c	2536499	Chocolate milk	*Bacillus toyonensis*	GJN427	Vegetal product	Spores	/	1,8
c	2536500	Milk coffee Latte classico	*Bacillus toyonensis*	GJN427	Vegetal product	Spores	/	1,8
c	2536501	Strawberry milk	*Bacillus toyonensis*	GJN427	Vegetal product	Spores	/	1,8
b	2664046	Organic millet drink	*Bacillus aerius*	KRPX57	Plant-based drink	Spores	/	4,4
b	2664047	Banana flavored soy drink	*Bacillus aerius*	KRPX57	Plant-based drink	Spores	/	1,6
b	2684344	Soy drink with sugar	*Bacillus thuringiensis*	GDX819	Grated coconut	Spores	/	2,1
b	2684346	Organic hemp drink	*Bacillus thuringiensis*	GDX819	Grated coconut	Spores	/	4,4

Verification of growth on salt-supplemented agar

Strain	Code strain	TSAYE (CFU/mL)	TSAYE (log CFU/mL)	TSAYE+salt (CFU/mL)	TSAYE+salt (log CFU/mL)	≠ log TSAYE/TSAYE+salt
Candida parapsilosis	GVNH48	7,0E+08	8,85	5,5E+08	8,74	0,10
Leuconostoc citreum	ADBW69	4,1E+08	8,61	3,1E+08	8,49	0,12
Micrococcus sp.	ADLA78	4,3E+07	7,63	2,7E+07	7,43	0,20
Saccharomyces cerevisiae	HQHR84	1,3E+07	7,11	8,6E+06	6,93	0,18
Rhodotorula mucilaginosa	FVYW49	5,5E+07	7,74	6,7E+07	7,83	-0,09
Stenotrophomonas maltophila	HWV749	3,8E+07	7,58	3,0E+07	7,48	0,10

Appendix D
Sensitivity study - raw results

Bacterial burden	*Distribution of flora*
Ø: no culture	A = pure culture of suspect colonies
L = low	B = mixture with a majority of suspect colonies
M = moderate	C = mixture with a minority of suspect colonies
H = high	D = mixture with rare suspect colonies
/: not realized	
	(x): x colonies characteristic of microrganism if x ≤ 5

PA: positive agreement
NA: negative agreement
ND: negative deviation
PD: positive deviation
PPNA: positive presumptive negative agreement
PPND : positive presumptive negative deviation
/: not realized

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 720 48 hours - 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
a+	2514883	Organic whole milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 75	7,5E+06	P	+	+ (AM)	*Nialla circulans (Bacillus circulans)*	P	PA
a+	2514884	Semi- skimmed milk 10 vitamins	pur -1 -2 -3 -4 -5	205 20 4 0 0 0	200	P	+	+ (AM)	*Enterococcus faecalis*	P	PA
a+	2514885	Semi- skimmed milk lactose free	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 >300	3,0E+07	P	+	+ (AM)	*Listeria innocua*	P	PA
a+	2514886	Skimmed milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 >300	3,0E+07	P	+	+ (AM)	*Listeria monocytogenes*	P	PA
a+	2514887	Semi-skimmed sheep's milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 >300	3,0E+07	P	+	+ (AM)	*Listeria monocytogenes*	P	PA
a+	2514921	Skimmed milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	+	+ (AH)	*Escherichia coli*	P	PD
a+	2514922	Semi-organic skimmed milk lactose-free	-2 -3 -4 -5 -6 -7	>300 >300 >300 187 19 3	1,9E+07	P	+	+ (AH)	*Staphylococcus aureus*	P	PA
a+	2514923	Semi-skimmed milk	-2 -3 -4 -5 -6 -7	>300 >300 >300 >300 58 7	5,9E+07	P	+	+ (AH)	*Staphylococcus aureus*	P	PA
a+	2514924	Semi-organic skimmed milk	-2 -3 -4 -5 -6 -7	>300 >300 >300 >300 >300 79	7,2E+08	P	+	+ (AH)	*Escherichia coli*	P	PA
a+	2514919	Semi-skimmed milk	-2 -3 -4 -5 -6 -7	>300 >300 >300 >300 36 4	3,6E+07	P	+	+ (AM)	*Bacillus cereus*	P	PA
a+	2514920	Semi-skimmed organic milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	+	+ (AM)	*Bacillus circulans*	P	PD
a+	2514917	Semi-skimmed milk	pur -1 -2 -3 -4 -5 -6 -7	>300 >300 >300 >300 >300 61 3 0	5,8E+06	P	+	+ (AM)	*Paenibacillus sp*	P	PA
a+	2514918	Goat's milk semi skimmed	-1 -2 -3 -4 -5 -6	>300 >300 >300 >300 34 8	3,8E+06	P	-	ø	/	A	ND
a+	2536424	Whole organic milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 206 41	2,3E+06	P	+	+ (AM)	*Candida parapsilosis*	P	PA
a+	2536425	Whole organic milk	-1 -2 -3 -4 -5 -6	>300 >300 >300 >300 >300 38	4,1E+06	P	+	+ (AM)	*Candida parapsilosis*	P	PA

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 720 48 hours - 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
a+	2536426	Semi-skimmed milk	-2 -3 -4 -5 -6 -7	>300 >300 >300 >300 56 13	6,3E+07	P	+	+ (AM)	*Leuconostoc citreum*	P	PA
a+	2536427	Whole organic milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 193 29	2,0E+06	P	+	+ (AM)	*Klebsiella pneumoniae*	P	PA
a+	2536428	Semi-skimmed sheep's milk	-2 -3 -4 -5 -6 -7	>300 >300 >300 >300 74 3	7,0E+07	P	+	+ (AM)	*Klebsiella pneumoniae*	P	PA
a-	2514908	Organic whole milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514909	Semi-skimmed milk 10 vitamins	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514910	Semi-skimmed milk lactose free	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514911	Skimmed milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514912	Semi-skimmed sheep's milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514948	Skimmed milk 0% FAT	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514949	Semi-organic skimmed milk lactose-free	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514950	Semi-skimmed milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514951	Semi-organic skimmed milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514952	Semi-organic skimmed milk lactose-free	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2514953	Organic semi-skimmed milk mountain milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
a-	2536436	Semi-skimmed milk	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 720 48 hours - 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
a-	2536437	Whole milk	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA
a-	2536438	Skimmed milk	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA
a-	2536439	Semi-skimmed organic milk	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA
c+	2514889	Chocolate milk	pur -1 -2 -3 -4 -5	>300 >300 50 7 2 0	5,2E+03	P	+	+ (AM)	*Nialla circulans (Bacillus circulans)*	P	PA
c+	2514891	Vanilla milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 >300	3,0E+07	P	+	+ (AM)	*Enterococcus faecalis*	P	PA
c+	2514892	Chocolate-caramel milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 >300	3,0E+07	P	+	+ (AM)	*Listeria innocua*	P	PA
c+	2514893	Strawberry milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 >300	3,0E+07	P	+	+ (AM)	*Listeria monocytogenes*	P	PA
c+	2514894	Chocolate milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 >300	3,0E+07	P	+	+ (AM)	*Listeria monocytogenes*	P	PA
c+	2514929	Chocolate milk	pur -1 -2 -3 -4 -5	0/0 0/0 0/0 0/0 0/0 0/0	<1	A	+	+ (AH)	*Escherichia coli*	P	PD
c+	2514931	Organic chocolate milk	-2 -3 -4 -5 -6 -7	>300 >300 >300 81 9 1	8,2E+06	P	+	+ (AH)	*Staphylococcus aureus*	P	PA
c+	2514932	Chocolate milk	-2 -3 -4 -5 -6 -7	>300 >300 >300 >300 >300 62	6,2E+08	P	+	+ (AH)	*Escherichia coli*	P	PA
c+	2514927	Coffe milk	pur -1 -2 -3 -4 -5	0/0 0/0 0/0 0/0 0/0 0/0	<1	A	+	+ (AM)	*Bacillus cereus*	P	PD
c+	2514928	Vanilla milk	-2 -3 -4 -5 -6 -7	>300 >300 34 2 0 0	3,3E+05	P	+	+ (AM)	*Bacillus circulans*	P	PA
c+	2514925	Hazelnut chocolate milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 >300	3,0E+07	P	+	+ (AM)	*Paenibacillus sp*	P	PA
c+	2536418	Milk with almond	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 107	1,1E+07	P	+	+ (AM)	*Candida parapsilosis*	P	PA

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 720 48 hours - 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
c+	2536419	Coffee with milk	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 143 5	1,4E+06	P	+	+ (AM)	*Leuconostoc citreum*	P	PA
c+	2536420	Strawberry milk	-2 -3 -4 -5 -6 -7	>300 >300 >300 >300 80 4	7,6E+07	P	+	+ (AM)	*Klebsiella pneumoniae*	P	PA
c+	2536421	Chocolate milk	-3 -4 -5 -6 -7 -8	>300 >300 >300 >300 25 3	2,5E+08	P	+	+ (AM)	*Klebsiella pneumoniae*	P	PA
c+	2536499	Chocolate milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	+	+ (AM)	*Bacillus toyonensis*	P	PD
c+	2536500	Milk coffee Latte classico	pur -1 -2 -3 -4 -5	>300 >300 >300 120 10 0	1,2E+05	P	-	ø	/	A	ND
c+	2536501	Strawberry milk	pur -1 -2 -3 -4 -5	>300 >300 >300 230 21 0	2,3E+05	P	-	ø	/	A	ND
c-	2514913	Chocolate milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
c-	2514914	Vanilla milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	+	ø/Bis: ø - Enum: <1 - BHI: PCA -/TSAYE - D-COUNT: -/-/- PCA anaér:- TSC anaér:-		A (FP)	NA (PP)
c-	2514915	Chocolate-caramel milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
c-	2514916	Strawberry milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
c-	2514930	Cappuccino milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
c-	2514954	Chocolate milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
c-	2514955	Chocolate milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
c-	2514956	Organic chocolate milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
c-	2514957	Chocolate milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 720 48 hours - 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
c-	2514958	Coffe milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	Ø	/	A	NA
c-	2514959	Vanilla milk	pur -1 -2 -3 -4 -5	1 0 0 0 0 0	1	A	-	Ø	/	A	NA
c-	2514926	Strawberry milk	pur -1 -2 -3 -4 -5	13 2 0 0 0 0	14	A	-	Ø	/	A	NA
c-	2536430	Milk coffee Latte cappuccino	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA
c-	2536431	Milk coffee Latte classico	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA
c-	2536432	Chocolate milk	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA
c-	2536433	Strawberry milk	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA
c-	2536434	Chocolate milk	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA
c-	2536435	Milk chocolate brownie flavor	pur -1 -2 -3 -4 -5	0 0 / / / /	<1	A	-	Ø	/	A	NA
c-	2536417	Chocolate milk	pur -1 -2 -3 -4 -5	0 0 0 0 0 /	<1	A	-	Ø	/	A	NA
c-	Supp. test 1	Vanilla milk (batch 1)	pur -1 -2 -3 -4 -5	0 0 0 0 0 /	<1	A	-	Ø	/	A	NA
c-	Supp. test 2	Vanilla milk (batch 2)	pur -1 -2 -3 -4 -5	0 0 0 0 0 /	<1	A	-	Ø	/	A	NA
c-	Supp. test 3	Vanilla milk (batch 3)	pur -1 -2 -3 -4 -5	0 0 0 0 0 /	<1	A	-	Ø	/	A	NA

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 714 72 hours at 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
b-	2514945	Oat drink ALPRO (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2514946	Almond and vanilla drink ALPRO (water + almond milk)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2514947	Roasted almond milk drink ALPRO (water + almond milk)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2498750	Oat drink ALPRO (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2498751	Hazelnut drink U (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2498752	Almond drink U (water)	pur -1 -2 -3 -4 -5	1 0 0 0 0 0	1	A	-	ø	/	A	NA
b-	2498753	Pea protein drink WUNDA (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2514960	Drink made from corn, oats, almonds SOJASUN (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2514961	Rice drink NAT ET VIE (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2514962	Macchiato flavored soy drink ALPRO (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2514963	Chocolate flavored hazelnut drink NAT ET VIE (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2514964	Coconut drink NAT ET VIE (water + coco cream)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2514965	Soy chocolate drink SOJASUN (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2514967	Soy drink free sugar SOJASUN (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 714 72 hours at 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
b-	2514969	Organic drink with oats and Lithothamnium algae LINA (water)	pur -1 -2 -3 -4 -5	1 0 0 / / /	1	A	-	ø	/	A	NA
b-	2514970	Organic cashew drink LA VIE CLAIRE (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b-	2514972	Organic drink made from cereals and nuts BJORG (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b-	2514973	Organic drink made from chestnut, rice and oats BJORG (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b-	2588500	Organic drink made from rice and vanilla ISOLA BIO (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b-	2588501	Organic spelled drink ISOLA BIO (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b-	2588502	Organic protein drink made from yellow peas BJORG (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b-	2588503	Organic almond cappuccino drink BJORG (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b-	2588504	Organic almond pea protein drink BJORG (water)	pur -1 -2 -3 -4 -5	14 0 0 / / /	14	A	-	ø	/	A	NA
b-	2588505	Vanilla-flavored plant-based drink made from peas WUNDA (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b-	2588506	Sugar-free vegetable drink made from peas WUNDA (water)	pur -1 -2 -3 -4 -5	1 0 0 / / /	1	A	-	ø	/	A	NA
b-	2588508	French soy drink with spelled and hazelnut SOY (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b-	2588509	Chocolate-flavored plant-based drink made from peas WUNDA (water)	pur -1 -2 -3 -4 -5	0 0 0 / / /	<1	A	-	ø	/	A	NA
b+	2588543	Oat drink (water)	-1 -2 -3 -4 -5 -6	>300 >300 >300 >300 >300 43	4,3E+07	P	+	+ AH	*Bacillus pumilus*	P	PA

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 714 72 hours at 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
b+	2588547	Drink made from corn, oats, almonds SOJASUN (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 >300	>3 10^7	P	-	ø	/	A	ND
b+	2588548	Rice drink NAT ET VIE (water)	-1 -2 -3 -4 -5 -6	>300 >300 >300 >300 177 22	1,8E+07	P	+	+ AH	*Bacillus pumilus*	P	PA
b+	2588552	Soy and chocolate drink SOJASUN (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	+	+ AM	*Micrococcus (Kocuria)*	P	PD
b+	2588553	Soy drink with sugar SOJASUN (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 75 10	7,7E+05	P	+	+ AH	*Bacillus pumilus*	P	PA
b+	2588557	Organic cashew drink LA VIE CLAIRE (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 43	4,3E+06	P	+	+ AL	*Micrococcus (Kocuria)*	P	PA
b+	2588560	Organic drink made from chestnut, rice and oats BJORG (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 87	8,7E+06	P	-	ø	/	A	ND
b+	2588561	Organic drink made from rice and vanilla ISOLA BIO (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 174 28 1	2,80E+05	P	-	ø	/	A	ND
b+	2588562	Organic spelled drink ISOLA BIO(water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 300	3,0E+07	P	+	+ AM	*Paenibacillus*	P	PA
b+	2588563	Organic protein drink made from yellow peas BJORG (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 40	4,0E+06	P	+	+ AM	*Paenibacillus*	P	PA
b-	2588564	Organic almond cappuccino drink BJORG (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b-	2588565	Organic almond pea protein drink BJORG (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b+	2588566	Vanilla-flavored plant-based drink made from peas WUNDA (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 20	2,0E+06	P	-	ø	/	A	ND
b+	2588567	Sugar-free plant-based drink made from peas WUNDA (water)	-1 -2 -3 -4 -5 -6	>300 >300 >300 >300 >300 29	2,9E+07	P	+	+ AM	*Saccharomyces cerevisiae*	P	PA
b+	2588540	Oat drink enriched with calcium and vitamin D ALPRO	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 44 7	4,6E+05	P	+	+ AL	*Rhodotorula mucilaginosa*	P	PA
b-	2588541	Vanilla flavored almond drink ALPRO (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b+	2588542	Roasted almond milk drink without sugar ALPRO (water)	-2 -3 -4 -5 -6 -7	>300 >300 >300 182 24 2	2,4E+07	P	+	+ AH	*Pantoea sp.*	P	PA

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 714 72 hours at 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
b+	2588544	Hazelnut drink U (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 109 11	1,1E+06	P	+	+ AH	*Bacillus cereus*	P	PA
b+	2588545	Almond drink U (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	+	+ AH	*Rhodotorula mucilaginosa*	P	PD
b+	2588546	Pea protein drink WUNDA (water)	-2 -3 -4 -5 -6 -7	>300 >300 >300 192 18 2	1,8E+07	P	+	+ AH	*Pantoea sp.*	P	PA
b-	2588549	Macchiato flavored soy drink ALPRO (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b+	2588550	Chocolate flavored hazelnut drink NAT ET VIE (water)	-1 -2 -3 -4 -5 -6	>300 >300 >300 >300 >300 80	8,0E+07	P	+	+ AH	*Pantoea sp.*	P	PA
b+	2588551	Coconut drink NAT ET VIE (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 124 12	1,2E+07	P	+	+ AL	*Rhodotorula mucilaginosa*	P	PA
b+	2588554	Plain soy drink without sugar SOJASUN (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 123 13	1,2E+06	P	+	+ AH	*Bacillus cereus*	P	PA
b-	2588555	Organic hemp drink ECOMIL (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b+	2588556	Organic drink with oats and Lithothamnium algae LINA (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 40	4,0E+06	P	+	+ AM	*Rhodotorula mucilaginosa*	P	PA
b+	2588558	Organic millet drink ISOLA BIO (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 260 32	2,7E+06	P	-	ø	/	A	ND
b+	2588559	Organic drink made from cereals and nuts BJORG (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 71	7,1E+06	P	+	+ AH	*Bacillus cereus*	P	PA
b-	2612499	Hazelnut drink BIO VILLAGE (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	A	-	ø	/	A	NA
b+	2615000	Spelled drink BIO VILLAGE (water)	-1 -2 -3 -4 -5 -6	>300 >300 >300 >300 47 6	4,8E+06	P	+	+ AH	*Pantoea sp.*	P	PA
b+	2612501	Oat drink BIO VILLAGE (water)	pur -1 -2 -3 -4 -5	>300 >300 130 11 1 0	1,3E+04	P	+	+ AM	*E. coli*	P	PA
b+	2612673	Coconut drink with vitamin B12 NAT ET VIE (water)	pur -1 -2 -3 -4 -5	>300 54 4 0 0 0	5,3E+02	P	+	+ AM	*Staphylococcus aureus*	P	PA

Type	#	Sample	ISO 4833-1▪				D-COUNT - Protocol 714 72 hours at 30°C				
			Dilution	UFC	Result	Final result	Result	Confirmation	Identification	Final result	Agreement
Supplementary tests including in the statistical interpretation											
b+	2664046	Organic millet drink ISOLA BIO (water)	pur -1 -2 -3 -4 -5	>300 53 7 1 0 0	540	**P**	+	+ AM	*Bacillus aerius*	**P**	**PA**
b+	2664047	Banana flavored soy drink ALPRO (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 68 6	6,8E+05	**P**	+	+ AM	*Bacillus aerius*	**P**	**PA**
b+	2684344	Soy drink with sugar SOJASUN (water)	pur -1 -2 -3 -4 -5	>300 >300 >300 >300 >300 131	1,3E+07	**P**	-	+ AM	*Bacillus cereus*	**P**	**PA**
b+	2684346	Organic hemp drink SOON (water)	pur -1 -2 -3 -4 -5	144 23 0 0 0 0	1,5E+03	**P**	-	+ AM	*Bacillus cereus*	**P**	**PA**
Samples excluding of the statistical interpretation											
b+	2514966	Soy drink with sugar SOJASUN (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	**A**	+	+ AH	*Bacillus altitudinis*	**P**	**PD**
b+	2514968	Organic hemp drink ECOMIL (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	**A**	+	+ AH	*Bacillus aerius*	**P**	**PD**
b+	2514971	Organic millet drink ISOLA BIO (water)	pur -1 -2 -3 -4 -5	1 0 0 0 0 0	1	**A**	+	+ AH	*Bacillus aerius*	**P**	**PD**
b+	2588507	Banana flavored soy drink ALPRO (water)	pur -1 -2 -3 -4 -5	0 0 0 0 0 0	<1	**A**	+	+ AH	*Bacillus thuringiensis*	**P**	**PD**

Appendix E
Relative Level of Detection - raw results

Strain	Product	Sample	Inoculation/ level	Reference method ISO 4833-1▪			D-COUNT 48h at 30°C			
				mPCA	Final result	Number positive samples/Total	D-COUNT	Confirmation	Final result	Number positive samples/Total
Stenotrophomonas maltophila HWV749 Stress applied: 10' at 56°C ≠log: 1.04	UHT milk	2612502	0	<1	A	0+/5	-	∅	A	0+/5
		2612503		<1	A		-	∅	A	
		2612504		<1	A		-	∅	A	
		2612505		<1	A		-	∅	A	
		2612506		<1	A		-	∅	A	
		2612507	0.9	>30 000	P	14+/20	-	∅	A	15+/20
		2612508		>30 000	P		-	∅	A	
		2612509		<1	A		+	+	P	
		2612510		>30 000	P		+	+	P	
		2612511		<1	A		+	+	P	
		2612512		<1	A		+	+	P	
		2612513		>30 000	P		+	+	P	
		2612514		>30 000	P		+	+	P	
		2612515		>30 000	P		-	∅	A	
		2612516		>30 000	P		+	+	P	
		2612517		>30 000	P		+	+	P	
		2612518		<1	A		+	+	P	
		2612519		>30 000	P		-	∅	A	
		2612520		>30 000	P		+	+	P	
		2612521		>30 000	P		+	+	P	
		2612522		<1	A		+	+	P	
		2612523		>30 000	P		+	+	P	
		2612524		<1	A		-	∅	A	
		2612525		>30 000	P		+	+	P	
		2612526		>30 000	P		+	+	P	
		2612527	2.8	>30 000	P	5+/5	+	+	P	5+/5
		2612528		>30 000	P		+	+	P	
		2612529		>30 000	P		+	+	P	
		2612530		>30 000	P		+	+	P	
		2612531		>30 000	P		+	+	P	

RLOD study - raw results - Plant-based drink

Strain	Product	Sample	Inoculation/ level	Reference method ISO 4833-1▪			D-COUNT 72h at 30°C			
				PCA	Final result	Number positive samples/Total	D-COUNT	Confirmation	Final result	Number positive samples/Total
Bacillus subtilis WJM246 sporulated form	Soy plant-based drink	2612532	0	<1	A	0+/5	-	∅	A	0+/5
		2612533		<1	A		-	∅	A	
		2612534		<1	A		-	∅	A	
		2612535		<1	A		-	∅	A	
		2612536		<1	A		-	∅	A	
		2612537	1.0	>30 000	P	12+/20	-	∅	A	12+/20
		2612538		<1	A		-	∅	A	
		2612539		>30 000	P		+	+	P	
		2612540		<1	A		+	+	P	
		2612541		<1	A		+	+	P	
		2612542		<1	A		+	+	P	
		2612543		>30 000	P		+	+	P	
		2612544		>30 000	P		-	∅	A	
		2612545		>30 000	P		+	+	P	
		2612546		>30 000	P		-	∅	A	
		2612547		>30 000	P		-	∅	A	
		2612548		<1	A		-	∅	A	
		2612549		<1	A		+	+	P	
		2612550		>30 000	P		+	+	P	
		2612551		<1	A		+	+	P	
		2612552		<1	A		+	+	P	
		2612553		>30 000	P		+	+	P	
		2612554		>30 000	P		-	∅	A	
		2612555		>30 000	P		+	+	P	
		2612556		>30 000	P		-	∅	A	
		2612557	2.0	>30 000	P	5+/5	+	+	P	4+/5
		2612558		>30 000	P		-	∅	A	
		2612559		>30 000	P		+	+	P	
		2612560		>30 000	P		+	+	P	
		2612561		>30 000	P		+	+	P	

Appendix F
Inclusivity study - raw results

Appendix F
Inclusivity study - Raw results

#	Strain	Form	Origin	Reference	Inoculation level	D-COUNT 48 hours		Comments
						Result	Confirmation	
1	*Aerococcus viridans*	Vegetative	Raw milk cheese	VAF957	34	+	+	/
2	*Bacillus cereus cereus*	Spore	Cheese	FXM250	34	+	+	/
3	*Bacillus cereus pseudomycoïdes*	Spore	Brocoli purée	FFT320	30	+	+	/
4	*Bacillus circulans*	Spore	Infant powder formula	CQND74	16	+	+	/
5	*Bacillus coagulans*	Spore	Evaporated milk	FNX731	12	+	+	/
6	*Bacillus licheniformis*	Spore	Milk powder	CZL399	33	+	+	/
7	*Bacillus pumilus*	Spore	Minth	DLX565	50	+	+	/
8	*Bacillus subtilis*	Spore	Mains industrial water	NBW594	21	-	-	Reference method: <1 CFU/ml
	Bacillus subtilis	Spore	Curcuma	WJM246	43	+	+	/
9	*Brevibacillus parabrevis*	Vegetative	Cheese	FND144	38	+	+	/
10	*Candida parapsilosis*	Vegetative	Dairy environment	GVNH48	96	+	+	/
11	*Corynebacterium flavescens*	Vegetative	Raw milk cheese	FBM018	49	+	+	/
12	*Enterobacter cloacae*	Vegetative	Lactoserum	VBT249	15	+	+	/
13	*Enterococcus faecalis*	Vegetative	Raw milk cheese	GCQ471	82	+	+	/
14	*Enterococcus faecium*	Vegetative	Raw milk cheese	WDF839	43	+	+	/
15	*Escherichia coli*	Vegetative	Raw milk cheese	TDW583	33	+	+	/
16	*Klebsiella oxytoca*	Vegetative	Dairy environment	UNG158	16	+	+	/
17	*Klebsiella pneumoniae*	Vegetative	Milk	CEC296	20	+	+	/
18	*Kluyvera cryocrescens*	Vegetative	Raw milk cheese	HNB846	32	+	+	/
19	*Lactococcus lactis*	Vegetative	Collection strain	WDCM0016	83	+	+	/
20	*Listeria innocua*	Vegetative	Pasteurized milk cheese	BLV059	52	+	+	/
21	*Listeria ivanovii*	Vegetative	Environment	GQD028	71	+	+	/
22	*Listeria monocytogenes*	Vegetative	Raw sheep's milk	12MOB096LM	22	+	+	/
23	*Lysinibacillus fusiformis*	Vegetative	Panna cotta with red fruits	BMH115	83	+	+	/
24	*Micrococcus luteus*	Vegetative	Pond water	NBV721	86	-	-	D-COUNT at 72h: + (3936) / PCA: + Pure strain: D-COUNT: + (>20 000) Reference method: <1 CFU/ml
	Micrococcus luteus	Vegetative	Water	SKZ371	24	-	-	Pure strain: D-COUNT: + (>20 000) Reference method: <1 CFU/ml
25	*Micrococcus sp.*	Vegetative	Vegetal extract	ADLA78	73	+	+	/
26	*Paenibacillus polymyxa*	Vegetative	Floor industry	FQJ653	11	+	+	/
27	*Pantoea agglomerans*	Vegetative	Udder of a cow	HUF873	19	+	+	/
28	*Raoultella ornithinolytica*	Vegetative	Pasteurized milk cheese	HFE309	15	+	+	/
29	*Staphylococcus aureus*	Vegetative	Raw milk cheese	EXX511	26	+	+	/
30	*Staphylococcus caprae*	Vegetative	Goat cheese	UJC759	36	+	+	/
31	*Staphylococcus equorum*	Vegetative	Raw milk cheese	WEN929	57	+	+	/
32	*Staphylococcus xylosus*	Vegetative	Raw milk cheese	TYR350	58	+	+	/
33	*Stenotrophomonas maltophila*	Vegetative	Milk cheese crust believed	BHL731	78	+	+	/
34	*Streptococcus thermophilus*	Vegetative	Infant powder formula	ESKW37	49	+	PCA 30°C: ∅	MRS 30°C: ∅ / PCA 44°C:+ / MRS 44°C: +
35	*Lactobacillus acidophilus*	Vegetative	Dairy product	HNSS85	17	+	+	/
36	*Lactobacillus paracasei*	Vegetative	Dairy product	HPJH54	81	+	+	/
37	*Levilactobacillus brevis*	Vegetative	Brewery	HPMD67	57	+	PCA: ∅	Growth only with M17 in anaerobic conditions at 30°C
38	*Saccharomyces cerevisiae*	Vegetative	Dairy industry	HQHR84	34	+	+	/
39	*Candida albicans*	Vegetative	Dairy industry	HSXZ45	44	+	+	/
40	*Candida krusei*	Vegetative	Dairy industry	HSYB23	90	+	+	/
41	*Candida kefyr*	Vegetative	Dairy industry	HTFK57	36	+	+	/
42	*Pseudomonas Aeruginosa*	Vegetative	UHT milk	HTMP35	21	+	+	/
43	*Salmonella spp* subsp Dublin	Vegetative	Raw milk cheese	ZDP683	28	+	+	/
44	*Proteus vulgaris*	Vegetative	Cheese	HVJP46	52	+	+	/
45	*Zygosacharomyces bailii*	Vegetative	Dairy product	HWTU22	36	+	+	/
46	*Acinetobacter baumanii*	Vegetative	Environment dairy industry	HXFZ26	57	+	+	/
47	*Heyndrickxia sporothermodurans*	Spore	UHT milk	JPH494	43	+	+	/
48	*Bacillus altitudinis*	Spore	Plant-based drink	GJP77	15	+	+	/
49	*Pseudomonas libanensis*	Vegetative	UHT milk	JJDH94	80	+	+	/
50	*Aeromonas sp.*	Vegetative	Water	NGL176	90	+	+	/

Appendix F

Inclusivity study - Raw results

Supplementary tests on *Micrococcus luteus* strain

#	Strain	Form	Origin	Reference	Inoculation level /1L pack	Time point	D-COUNT		Enumeration mPCA CFU/ml	pH of the pack	Reference method
							Result	Confirmation			
24	*Micrococcus luteus*	Vegetative	Pond water	NBV721	92	24h	-	-	<1	6,75	/
						48h	-	-	<1	6,76	/
						5 days	-	-	5 CFU/ml	6,75	/
						6 days	-	-	45 CFU/ml	6,67	/
						8 days	+	+ (AM)	140 000 CFU/ml	6,76	/
						9 days	+	+ (AM)	2 900 000 CFU/ml	6,70	/
						13 days	+	+ (AM)	13 000 000 CFU/ml	6,74	/
						14 days	+	+ (AM)	24 000 000 CFU/ml	6,70	/
						15 days	+	+ (AM)	21 000 000 CFU/ml	6,67	21 000 000 CFU/ml

Appendix G: Interlaboratory study - raw results obtained by Expert Lab

microsept VALIDATION & EXPERTISE Send by email at g.mesnard@microsept.fr			Interlaboratory study for MicroVal certification of the D-COUNT method				
			Name of laboratory:		Name and visa of the person responsible for the analyzes:		
			Microsept				
ISO 4833-1*					D-COUNT		
Laboratory code	Sample code	15 days 30°C + PCA+lait 72h at 30°C			Sample code	48 hours at 30°C	
Letter	n°	Dilutions	Number of CFU/plate	Result	n°	D-COUNT Result	
Expert Lab	25	pur	0	Negative	1	Positive >20 000	
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Expert Lab	26	pur	0	Negative	2	Negative	
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Expert Lab	27	pur	>300	Positive	3	Positive >20 000	
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	>300				
Expert Lab	28	pur	>300	Positive	4	Positive >20 000	
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	>300				
Expert Lab	29	pur	0	Negative	5	Negative	
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Expert Lab	30	pur	>300	Positive	6	Positive >20 000	
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	>300				
Expert Lab	31	pur	0	Negative	7	Negative	
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Expert Lab	32	pur	>300	Positive	8	Positive >20 000	
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	>300				
Expert Lab	33	pur	0	Negative	9	Negative	
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Expert Lab	34	pur	>300	Positive	10	Negative	
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	>300				
Expert Lab	35	pur	>300	Positive	11	Positive >20 000	
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	>300				

ISO 4833-1*					D-COUNT	
Laboratory code	Sample code	15 days 30°C + PCA+lait 72h at 30°C			Sample code	48 hours at 30°C
Letter	n°	Dilutions	Number of CFU/plate	Result	n°	D-COUNT Result
Expert Lab	36	pur	0	Negative	12	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Expert Lab	37	pur	>300	Positive	13	Positive >20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Expert Lab	38	pur	0	Negative	14	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Expert Lab	39	pur	>300	Positive	15	Positive >20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Expert Lab	40	pur	0	Negative	16	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Expert Lab	41	pur	>300	Positive	17	Positive >20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Expert Lab	42	pur	>300	Positive	18	Positive >20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Expert Lab	43	pur	0	Negative	19	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Expert Lab	44	pur	0	Negative	20	Positive >20 000
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Expert Lab	45	pur	>300	Positive	21	Positive >20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Expert Lab	46	pur	>300	Positive	22	Positive >20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Expert Lab	47	pur	0	Negative	23	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Expert Lab	48	pur	>300	Positive	24	Positive >20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
					Positive control	Positive
					Negative contro	Negative

Appendix H: Interlaboratory study - raw results obtained by the collaborators

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		Name of laboratory:		Name and visa of the person responsible for the analyzes:		
ISO 4833-1					D-COUNT	
Laboratory code	Sample code	15 days 30°C + PCA+lait 72h at 30°C			Sample code	48 hours at 30°C
Letter	n°	Dilutions	Number of CFU/plate	Result	n°	D-COUNT Result
Lab A	25	pur	> 300	Positive	1	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	26	pur	> 100	Négatif	2	Négatif
		1/10ème	> 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab A	27	pur	> 300	Positive	3	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	28	pur	> 300	Positive	4	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	29	pur	≤ 100	Négatif	5	Négatif
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab A	30	pur	> 300	Positive	6	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	31	pur	> 300	Positive	7	Négatif
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	32	pur	> 300	Positive	8	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	33	pur	≤ 100	Négatif	9	Négatif
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab A	34	pur	≤ 100	Négatif	10	Négatif
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab A	35	pur	> 300	Positive	11	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			

ISO 4833-1					D-COUNT	
Laboratory code	Sample code	15 days 30°C + PCA+lait 72h at 30°C			Sample code	48 hours at 30°C
Letter	n°	Dilutions	Number of CFU/plate	Result	n°	D-COUNT Result
Lab A	36	pur	≤ 100	Négatif	12	Négatif
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab A	37	pur	> 300	Positive	13	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	38	pur	≤ 100	Négatif	14	Négatif
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab A	39	pur	> 300	Positive	15	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	40	pur	≤ 100	Négatif	16	Négatif
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab A	41	pur	> 300	Positive	17	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	42	pur	> 300	Positive	18	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	43	pur	≤ 100	Négatif	19	Négatif
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab A	44	pur	> 300	Positive	20	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	45	pur	> 300	Positive	21	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	46	pur	> 300	Positive	22	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab A	47	pur	≤ 100	Négatif	23	Négatif
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab A	48	pur	> 300	Positive	24	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
					Positive control	Positive
					Negative contro	Negative

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ISO 4833-1					D-COUNT	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab B	25	pur	> 300	Positive	1	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	26	pur	≤ 100	Negative	2	Negative
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab B	27	pur	> 300	Positive	3	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	28	pur	> 300	Positive	4	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	29	pur	≤ 100	Negative	5	Negative
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab B	30	pur	> 300	Positive	6	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	31	pur	> 300	Positive	7	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	32	pur	> 300	Positive	8	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	33	pur	≤ 100	Negative	9	Negative
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab B	34	pur	≤ 100	Negative	10	Positive
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab B	35	pur	> 300	Positive	11	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	36	pur	≤ 100	Negative	12	Negative
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			

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Name of laboratory:	Name and visa of the person responsible for the analyzes:

ISO 4833-1					D-COUNT	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab B	37	pur	> 300	Positive	13	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	38	pur	≤ 100	Negative	14	Negative
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab B	39	pur	> 300	Positive	15	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	40	pur	≤ 100	Negative	16	Negative
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab B	41	pur	> 300	Positive	17	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	42	pur	> 300	Positive	18	Negative
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	43	pur	≤ 100	Negative	19	Negative
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab B	44	pur	≤ 100	Negative	20	Negative
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
Lab B	45	pur	> 300	Positive	21	Negative
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	46	pur	> 300	Positive	22	Positive
		1/10ème	> 300			
		1/100ème	> 300			
		1/1000ème	> 300			
Lab B	47	pur	> 100	Negative	23	Negative
		1/10ème	> 100			
		1/100ème	> 100			
		1/1000ème	> 100			
Lab B	48	pur	≤ 100	Negative	24	Positive
		1/10ème	≤ 100			
		1/100ème	≤ 100			
		1/1000ème	≤ 100			
					Positive control	Positive
					Negative contro	Negative

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			Name of laboratory:		Name and visa of the person responsible for the analyzes:	
					Manuel Martin	
ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab C	25	pur	INCONTABLE	Positive	1	0
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	26	pur	0	Negative	2	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab C	27	pur	INCONTABLE	Positive	3	0
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	28	pur	0	Negative	4	0
		1/10ème	18			
		1/100ème	0			
		1/1000ème	0			
Lab C	29	pur	0	Negative	5	0
		1/10ème	0			
		1/100ème	60			
		1/1000ème	5			
Lab C	30	pur	INCONTABLE	Positive	6	30
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	31	pur	INCONTABLE	Positive	7	0
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	32	pur	INCONTABLE	Positive	8	10
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	33	pur	0	Negative	9	0
		1/10ème	0			
		1/100ème	30			
		1/1000ème	60			
Lab C	34	pur	0	Negative	10	10
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab C	35	pur	INCONTABLE	Positive	11	10
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	36	pur	INCONTABLE	Positive	12	0
		1/10ème	INCONTABLE			
		1/100ème	60			
		1/1000ème	52			

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Interlaboratory study for MicroVal certification of the D-COUNT method

Name of laboratory:

Name and visa of the person responsible for the analyzes: Manuel Martin

ISO 4833-1					D-COUNT	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab C	37	pur	INCONTABLE	Positive	13	10
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	38	pur	84	Negative	14	0
		1/10ème	60			
		1/100ème	28			
		1/1000ème	2			
Lab C	39	pur	INCONTABLE	Positive	15	180
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	40	pur	0	Negative	16	0
		1/10ème	60			
		1/100ème	6			
		1/1000ème	2			
Lab C	41	pur	INCONTABLE	Positive	17	20
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	42	pur	50	Negative	18	0
		1/10ème	50			
		1/100ème	10			
		1/1000ème	1			
Lab C	43	pur	0	Negative	19	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab C	44	pur	INCONTABLE	Positive	20	0
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	45	pur	INCONTABLE	Positive	21	0
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	46	pur	INCONTABLE	Positive	22	10
		1/10ème	INCONTABLE			
		1/100ème	INCONTABLE			
		1/1000ème	INCONTABLE			
Lab C	47	pur	INCONTABLE	Positive	23	0
		1/10ème	INCONTABLE			
		1/100ème	80			
		1/1000ème	12			
Lab C	48	pur	0	Negative	24	10
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
					Positive control	Positive
					Negative contro	Negative

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	Name of laboratory:				Name and visa of the person responsible for the analyzes:	
ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab D	25	pur	INC	Positive	1	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	26	pur	11	Negative	2	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab D	27	pur	INC	Positive	3	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	28	pur	INC	Positive	4	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	29	pur	10	Negative	5	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab D	30	pur	INC	Positive	6	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	31	pur	INC	Positive	7	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	32	pur	INC	Positive	8	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	33	pur	0	Negative	9	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab D	34	pur	INC	Positive	10	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	35	pur	INC	Positive	11	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	36	pur	1	Negative	12	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			

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	Name of laboratory:				Name and visa of the person responsible for the analyzes:	
ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab D	37	pur	INC	Positive	13	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	38	pur	3	Negative	14	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab D	39	pur	INC	Positive	15	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	40	pur	13	Negative	16	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab D	41	pur	INC	Positive	17	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	42	pur	5	Negative	18	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab D	43	pur	0	Negative	19	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab D	44	pur	INC	Positive	20	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	45	pur	INC	Positive	21	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	46	pur	INC	Positive	22	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab D	47	pur	4	Negative	23	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab D	48	pur	INC	Positive	24	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
					Positive control	Positive
					Negative contro	Negative

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	Name of laboratory:				Name and visa of the person responsible for the analyzes:	
ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab E	25	pur	INC	Positive	1	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	26	pur	8	Negative	2	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab E	27	pur	INC	Positive	3	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	28	pur	INC	Positive	4	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	29	pur	8	Negative	5	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab E	30	pur	INC	Positive	6	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	31	pur	INC	Positive	7	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	32	pur	INC	Positive	8	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	33	pur	1	Negative	9	Negative
		1/10ème	4			
		1/100ème	0			
		1/1000ème	0			
Lab E	34	pur	INC	Positive	10	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	35	pur	INC	Positive	11	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	36	pur	0	Negative	12	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			

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Interlaboratory study for MicroVal certification of the D-COUNT method

Name of laboratory:

Name and visa of the person responsible for the analyzes:

ISO 4833-1					D-COUNT	
Laboratory code	Sample code	15 days 30°C + PCA+lait 72h at 30°C			Sample code	48 hours at 30°C
Letter	n°	Dilutions	Number of CFU/plate	Result	n°	D-COUNT Result
Lab E	37	pur	INC	Positive	13	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	38	pur	0	Negative	14	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab E	39	pur	INC	Positive	15	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	40	pur	0	Negative	16	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab E	41	pur	INC	Positive	17	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	42	pur	INC	Positive	18	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	43	pur	5	Negative	19	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab E	44	pur	INC	Positive	20	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	45	pur	INC	Positive	21	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	46	pur	INC	Positive	22	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
Lab E	47	pur	0	Negative	23	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab E	48	pur	INC	Positive	24	>20000
		1/10ème	INC			
		1/100ème	INC			
		1/1000ème	INC			
					Positive control	Positive
					Negative contro	Negative

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ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab F	25	pur	>300	Positive	1	Negative
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	26	pur	0	Negative	2	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab F	27	pur	>300	Positive	3	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	28	pur	>300	Positive	4	Negative
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	29	pur	0	Negative	5	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab F	30	pur	>300	Positive	6	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	31	pur	>300	Positive	7	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	32	pur	>300	Positive	8	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	33	pur	0	Negative	9	Negative
		1/10ème	0			
		1/100ème	64			
		1/1000ème	49			
Lab F	34	pur	>300	Positive	10	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	35	pur	>300	Positive	11	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	36	pur	76	Negative	12	Negative
		1/10ème	41			
		1/100ème	30			
		1/1000ème	9			

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Name of laboratory:	Name and visa of the person responsible for the analyzes:

ISO 4833-1					D-COUNT	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab F	37	pur	>300	Positive	13	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	38	pur	1	Negative	14	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab F	39	pur	>300	Positive	15	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	40	pur	26	Negative	16	Negative
		1/10ème	2			
		1/100ème	1			
		1/1000ème	0			
Lab F	41	pur	>300	Positive	17	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	42	pur	0	Negative	18	Positive
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab F	43	pur	0	Negative	19	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab F	44	pur	>300	Positive	20	Negative
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	45	pur	2	Negative	21	Positive
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab F	46	pur	>300	Positive	22	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab F	47	pur	2	Negative	23	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab F	48	pur	>300	Positive	24	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
					Positive control	Positive
					Negative contro	Negative

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ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab G	25	pur	>300	Positive	1	Negative
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	240			
Lab G	26	pur	0	Negative	2	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab G	27	pur	>300	Positive	3	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab G	28	pur	>300	Positive	4	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab G	29	pur	0	Negative	5	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab G	30	pur	>300	Positive	6	Positive
		1/10ème	>300			
		1/100ème	280			
		1/1000ème	28			
Lab G	31	pur	>300	Positive	7	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	130			
Lab G	32	pur	>300	Positive	8	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	260			
Lab G	33	pur	0	Negative	9	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab G	34	pur	0	Negative	10	Positive
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab G	35	pur	>300	Positive	11	>20000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	100			
Lab G	36	pur	0	Negative	12	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			

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ISO 4833-1					D-COUNT	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab G	37	pur	>300	Positive	13	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	70			
Lab G	38	pur	0	Negative	14	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab G	39	pur	>300	Positive	15	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	140			
Lab G	40	pur	0	Negative	16	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab G	41	pur	>300	Positive	17	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	160			
Lab G	42	pur	>300	Positive	18	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	50			
Lab G	43	pur	0	Negative	19	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab G	44	pur	>300	Positive	20	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	90			
Lab G	45	pur	0	Negative	21	Positive
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab G	46	pur	>300	Positive	22	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab G	47	pur	0	Negative	23	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab G	48	pur	1	Negative	24	Positive
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
					Positive control	Positive
					Negative contro	Negative

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ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab H	25	pur	>300	Positive	1	0
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	26	pur	0	Negative	2	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab H	27	pur	>300	Positive	3	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	28	pur	>300	Positive	4	0
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	29	pur	0	Negative	5	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab H	30	pur	>300	Positive	6	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	31	pur	>300	Positive	7	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	32	pur	>300	Positive	8	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	68000			
Lab H	33	pur	0	Negative	9	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab H	34	pur	>300	Positive	10	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	35	pur	>300	Positive	11	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	36	pur	0	Negative	12	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			

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Name and visa of the person responsible for the analyzes:
E.SAGOT

ISO 4833-1					D-COUNT	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab H	37	pur	>300	Positive	13	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	252			
Lab H	38	pur	0	Negative	14	20
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab H	39	pur	>300	Positive	15	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	40	pur	0	Negative	16	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab H	41	pur	>300	Positive	17	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	42	pur	>300	Positive	18	0
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	116			
Lab H	43	pur	0	Negative	19	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab H	44	pur	>300	Positive	20	0
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab H	45	pur	0	Negative	21	Positive
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab H	46	pur	>300	Positive	22	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	177			
Lab H	47	pur	0	Negative	23	10
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab H	48	pur	>300	Positive	24	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
					Positive control	Positive
					Negative contro	Negative

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						N.CAILLY	
ISO 4833-1						D-COUNT	
Laboratory code	Sample code	15 days 30°C + PCA+lait 72h at 30°C				Sample code	48 hours at 30°C
Letter	n°	Dilutions	Number of CFU/plate	Result		n°	D-COUNT Result
Lab I	25	pur	0	Negative		1	>20 000
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Lab I	26	pur	0	Negative		2	0
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Lab I	27	pur	>300	Positive		3	>20 000
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	188				
Lab I	28	pur	0	Negative		4	>20 000
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Lab I	29	pur	0	Negative		5	0
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Lab I	30	pur	>300	Positive		6	>20 000
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	>300				
Lab I	31	pur	>300	Positive		7	0
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	268				
Lab I	32	pur	>300	Positive		8	>20 000
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	184				
Lab I	33	pur	0	Negative		9	0
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Lab I	34	pur	0	Negative		10	0
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				
Lab I	35	pur	>300	Positive		11	>20 000
		1/10ème	>300				
		1/100ème	>300				
		1/1000ème	>300				
Lab I	36	pur	0	Negative		12	0
		1/10ème	0				
		1/100ème	0				
		1/1000ème	0				

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ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab I	37	pur	>300	Positive	13	>20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab I	38	pur	0	Negative	14	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab I	39	pur	>300	Positive	15	>20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab I	40	pur	0	Negative	16	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab I	41	pur	>300	Positive	17	>20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab I	42	pur	0	Negative	18	>20 000
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab I	43	pur	0	Negative	19	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab I	44	pur	>300	Positive	20	>20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab I	45	pur	>300	Positive	21	>20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	188			
Lab I	46	pur	>300	Positive	22	>20 000
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	144			
Lab I	47	pur	0	Negative	23	0
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab I	48	pur	>300	Positive	24	0
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
					Positive control	Positive
					Negative contro	Negative

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Name of laboratory:	Name and visa of the person responsible for the analyzes:

ISO 4833-1					D-COUNT	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab J	25	pur			1	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	26	pur			2	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	27	pur			3	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	28	pur			4	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	29	pur			5	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	30	pur			6	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	31	pur			7	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	32	pur			8	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	33	pur			9	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	34	pur			10	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	35	pur			11	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	36	pur			12	
		1/10ème				
		1/100ème				
		1/1000ème				

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ISO 4833-1					D-COUNT	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab J	37	pur			13	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	38	pur			14	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	39	pur			15	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	40	pur			16	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	41	pur			17	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	42	pur			18	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	43	pur			19	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	44	pur			20	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	45	pur			21	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	46	pur			22	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	47	pur			23	
		1/10ème				
		1/100ème				
		1/1000ème				
Lab J	48	pur			24	
		1/10ème				
		1/100ème				
		1/1000ème				
					Positive control	
					Negative control	

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ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab K	25	pur	>300	Positive	1	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	26	pur	0	Negative	2	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab K	27	pur	>300	Positive	3	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	28	pur	>300	Positive	4	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	29	pur	0	Negative	5	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab K	30	pur	>300	Positive	6	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	31	pur	>300	Positive	7	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	32	pur	>300	Positive	8	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	33	pur	0	Negative	9	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab K	34	pur	>300	Positive	10	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	35	pur	>300	Positive	11	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	36	pur	0	Negative	12	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			

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Interlaboratory study for MicroVal certification of the D-COUNT method

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ISO 4833-1					D-COUNT	
Laboratory code	Sample code	15 days 30°C + PCA+lait 72h at 30°C			Sample code	48 hours at 30°C
Letter	n°	Dilutions	Number of CFU/plate	Result	n°	D-COUNT Result
Lab K	37	pur	>300	Positive	13	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	38	pur	0	Negative	14	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab K	39	pur	>300	Positive	15	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	40	pur	4	Negative	16	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab K	41	pur	>300	Positive	17	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	42	pur	0	Negative	18	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab K	43	pur	0	Negative	19	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab K	44	pur	29	Negative	20	Positive
		1/10ème	4			
		1/100ème	0			
		1/1000ème	1			
Lab K	45	pur	>300	Positive	21	Negative
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	46	pur	>300	Positive	22	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab K	47	pur	0	Negative	23	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab K	48	pur	1	Negative	24	Positive
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
					Positive control	Positive
					Negative contro	Negative

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ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab L	25	pur	2	**Negative**	1	**Positive**
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab L	26	pur	1	**Retest sample= negative**	2	**Negative**
		1/10ème	0			
		1/100ème	>300			
		1/1000ème	1			
Lab L	27	pur	>300	**Positive**	3	**Positive**
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	28	pur	>300	**Positive**	4	**Negative**
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	29	pur	34	**Retest sample= negative**	5	**Negative**
		1/10ème	0			
		1/100ème	0			
		1/1000ème	>300			
Lab L	30	pur	>300	**Positive**	6	**Positive**
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	31	pur	1	**Negative**	7	**Negative**
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab L	32	pur	>300	**Positive**	8	**Positive**
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	33	pur	0	**Retest sample= negative**	9	**Negative**
		1/10ème	37			
		1/100ème	0			
		1/1000ème	7			
Lab L	34	pur	0	**Negative**	10	**Negative**
		1/10ème	1			
		1/100ème	1			
		1/1000ème	0			
Lab L	35	pur	>300	**Positive**	11	**Positive**
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	36	pur	0	**Negative**	12	**Negative**
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			

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Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab L	37	pur	>300	Positive	13	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	38	pur	8	Negative	14	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab L	39	pur	>300	Positive	15	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	40	pur	0	Negative	16	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab L	41	pur	>300	Positive	17	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	42	pur	>300	Positive	18	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	43	pur	0	Negative	19	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab L	44	pur	0	Negative	20	Positive
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab L	45	pur	>300	Positive	21	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	46	pur	>300	Positive	22	Positive
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
Lab L	47	pur	0	Negative	23	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab L	48	pur	>300	Positive	24	Negative
		1/10ème	>300			
		1/100ème	>300			
		1/1000ème	>300			
					Positive control	Positive
					Negative control	Negative

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			Name of laboratory:		Name and visa of the person responsible for the analyzes:	
					Valérie Rabouin - VR	
ISO 4833-1					**D-COUNT**	
Laboratory code	**Sample code**	**15 days 30°C + PCA+lait 72h at 30°C**			**Sample code**	**48 hours at 30°C**
Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab M	25	pur	0	Negative	1	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab M	26	pur	0	Negative	2	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab M	27	pur	TNTC	Positive	3	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	28	pur	TNTC	Positive	4	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	29	pur	0	Negative	5	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab M	30	pur	TNTC	Positive	6	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	31	pur	TNTC	Positive	7	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	32	pur	0	Negative	8	Positive
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab M	33	pur	0	Negative	9	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab M	34	pur	TNTC	Positive	10	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	35	pur	TNTC	Positive	11	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	36	pur	0	Negative	12	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			

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Letter	**n°**	**Dilutions**	**Number of CFU/plate**	**Result**	**n°**	**D-COUNT Result**
Lab M	37	pur	TNTC	Positive	13	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	38	pur	0	Negative	14	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab M	39	pur	TNTC	Positive	15	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	40	pur	0	Negative	16	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab M	41	pur	TNTC	Positive	17	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	42	pur	TNTC	Positive	18	Negative
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	43	pur	0	Negative	19	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab M	44	pur	TNTC	Positive	20	Negative
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	45	pur	TNTC	Positive	21	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	46	pur	TNTC	Positive	22	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab M	47	pur	1	Negative	23	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab M	48	pur	TNTC	Positive	24	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
					Positive control	Positive
					Negative contro	Negative

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		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	26	pur	0	Negative	2	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab N	27	pur	TNTC	Positive	3	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	28	pur	TNTC	Positive	4	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	29	pur	0	Negative	5	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab N	30	pur	TNTC	Positive	6	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	31	pur	0	Negative	7	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab N	32	pur	TNTC	Positive	8	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	33	pur	0	Negative	9	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab N	34	pur	TNTC	Positive	10	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	35	pur	TNTC	Positive	11	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	36	pur	0	Negative	12	Negative
		1/10ème	0			
		1/100ème	0			
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		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	38	pur	0	Negative	14	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab N	39	pur	TNTC	Positive	15	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	40	pur	0	Negative	16	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab N	41	pur	TNTC	Positive	17	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	42	pur	TNTC	Positive	18	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	43	pur	0	Negative	19	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab N	44	pur	TNTC	Positive	20	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	45	pur	TNTC	Positive	21	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	46	pur	TNTC	Positive	22	Positive
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
Lab N	47	pur	0	Negative	23	Negative
		1/10ème	0			
		1/100ème	0			
		1/1000ème	0			
Lab N	48	pur	TNTC	Positive	24	Negative
		1/10ème	TNTC			
		1/100ème	TNTC			
		1/1000ème	TNTC			
					Positive control	Positive
					Negative contro	Negative

IDENTIFICATION DE 9 SOUCHES PAR AMPLIFICATION ET SEQUENCAGE DE LA REGION 16S OU ITS

Client : LABORATOIRE MICROSEPT – 15 Rue Denis Papin 49220 LE LION D'ANGERS

Contact :

Email : analyses.specifiques@microsept.fr

Service **: Identification de 9 Souches : par Amplification 16S ou ITS et Séquençage Sanger**

Date de réception des Boites de Pétri avec colonies : **Le 22/10/2024 - Votre envoi du 21/10/2024**

Réfèrent Technique **: Mme Farida MEBARKI**

PROTOCOLE DE L'ANALYSE

1- Extraction de l'ADN génomique à partir des colonies sur gélose

2- Amplification de la région 16S ou ITS avec les amorces universelles

3- Contrôle Qualité de ces produits PCR sur QIAXCEL pour validation qualité et quantité de produit PCR (Une seule bande bien spécifique de taille identique à la taille attendue)

4- Purification de ces produits PCRs (purification par méthode Exosap)

5- Séquençage de ces produits PCRs avec les amorces externes et/ou des amorces internes

6- Analyse et Alignement des séquences pour créer un Contig de la séquence PCR complète

7- Blast sur 2 Bases de données publiques pour identification des souches analysées

CONDITIONS DES AMPLIFICATIONS PCR

Les conditions de la PCR sont:

Pour la réalisation des Mix :

Extraits ADNg : 2µl
Mix PCR 5X: 6µl
Primer For: 1µl
Primer Rev : 1µl
H2O qsp 30µl : 20 µl

Cycles de PCR : 96°C 12 min (activation du Mix HS)

Puis 35 cycles de 96°C 20sec- 56°C 20sec- 72°C 1min30
Extension finale à 72°C – 5 min

Analyse sur QIAXCEL des PCR
Un Contrôle Négatif (H2O) est systématiquement amplifié dans les mêmes conditions que le/les ADN à analyser

Microsynth

PURIFICATION DES AMPLICONS OBTENUS ET MISE EN SEQUENCAGE

Purification des amplicons avec le kit **ExoSap-It (USB)**
Les produits PCR sont séquençés avec le kit **BigDyeV3.1 Dye Terminator Kit** (Thermo Fisher Scientific).

Les réactions de séquence sont ensuite purifiées par précipitation à l'éthanol avant analyse sur un **séquenceur 96 capillaires ABI3730XL**

RESULTATS DES ANALYSES Blast

SOUCHE	*RESULTAT IDENTIFICATION*	*% d'identité*	*score*
	BACTERIES		
VDM-L33-10^{-3}	Streptococcus_salivarius	99.87%	2724
VDM-L33-10^{-1}	Staphylococcus_epidermidis	99.93%	2713
VDM-L29-10^{-3}	Streptococcus_salivarius	99.87%	2724
VDM-L26-10^{-2}	Staphylococcus_epidermidis	99.93%	2713
VDM-L26-PUR	Staphylococcus_epidermidis	99.93%	2713

Rapport d'Analyse établi à Vaulx-en-Velin : le 31 Octobre 2024

MEBARKI Farida

MICROSYNTH France sas
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170, Avenue Gabriel Péri 69120 Vaulx en Velin
Tél : 04 37 45 02 96
Email : info@microsynth.fr
Sas au Capital de 500 000 €
Siret : 900 229 352 00019