

NF VALIDATION 16140™

AFNOR CERTIFICATION VALIDATION OF THE METHOD

GENE-UP *Listeria monocytogenes* (Ref. 414058)

Attestation number: BIO 12/40 – 11/16

For the detection of *Listeria monocytogenes*

Protocol for a broad range of foods and environmental samples

SUMMARY REPORT – MARCH 2017 – V1

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1. [Introduction](#)

This document introduces the study results for the AFNOR Certification validation of the GENE-UP *Listeria monocytogenes* method for the detection of *Listeria monocytogenes* in all human food products and environmental samples.

The GENE-UP *Listeria monocytogenes* method has been validated in November 2016 for the categories “Meat products” and “Dairy products”. An extension study concerning the addition of several categories to the application scope of the method has been validated in January 2017.

Assays were performed according to the standard ISO 16140-2: 2016 and to the *Requirements regarding comparison and interlaboratory studies for implementation of the standard EN ISO 16140-2, v5.1* (November 2015).

1.1. [Validation repository](#)

The aim of this summary report is to present the performance of the GENE-UP *Listeria monocytogenes* method regarding the reference method EN ISO 11290-1/A1 for all human food products and environmental samples according to the standard ISO 16140-2: 2016.

The validation study consisted in:

- a method comparison study which allowed determining the following parameters:
 - the sensitivity of the alternative method,
 - the relative level of detection of the alternative method,
 - the inclusivity and exclusivity of the method,
 - the practicability of the method.

- an interlaboratory study to compare the performance of the alternative method to the reference method under reproducibility conditions.

1.2. [Alternative method](#)

1.2.1. [Principle of the method](#)

The GENE-UP *Listeria monocytogenes* kit is to be used with compatible PCR strip tubes in the GENE-UP Thermocycler. Each reaction vial in the GENE-UP *Listeria monocytogenes* kit contains all of the necessary components for PCR, including sample-specific primers and probes and an internal amplification control.

The GENE-UP Thermocycler detects fluorescence at several wavelengths (channels) to allow for multi-target detection in the same reaction vessel. The fluorescent signal from a sample is recorded in channel 640, while the fluorescent signal for an internal amplification control is recorded in channel 705. The software automatically interprets the results for the internal amplification control and determines the sample result based on the outcome of the control.

Both the assay for the sample and the internal amplification control utilize dual Fluorescence Resonance Energy Transfer (FRET) hybridization probes. These probes consist of two different short oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the reaction cycle. The first probe for the sample assay is labeled at the 3' end with fluorescein; the second probe is labeled at the 5' end with LC Red 640. FRET occurs only after the two probes come in close proximity from hybridizing to the template DNA.

The resulting fluorescent signal from the FRET interaction, which forms a real-time amplification curve, is how the amplified target is detected by the GENE-UP Thermocycler. After the PCR cycling program finishes, the PCR

product(s) are melted to determine the presence of the target DNA. The software uses both the real-time amplification curve and the melt peak to make a positive or negative call.

The internal amplification control, contained in the reconstitution buffer, validates that the reaction conditions are sustainable for PCR to take place, thus validating a negative outcome for the sample. The internal amplification control is amplified by the same primer set but uses a different set of hybridization FRET probes to allow detection in the 705 channel.

1.2.2. [Protocol of the alternative method](#)

Two protocols are available for the alternative method:

- for food products and environmental samples, except surface samples: one-step enrichment of a test portion of 25 g in 225 mL of LPT broth* at 37±1°C for 25±3 h,

- for environmental surface samples: one-step enrichment of a swipe or sponge in 100 mL of LPT broth* at 37±1°C for 21±3 h, one-step enrichment of a swab in 10 mL of LPT broth at 37±1°C for 21±3 h.

*: The LPT broth must be pre-warmed at room temperature (15-25°C) before use.

The enrichment is followed by a lysis step and a detection step in the GENE-UP thermocycler.

Results are shown in the software Gene-Up Routine.

Presumptive positive results are confirmed by a streaking of 10 µL of the enriched LPT broth on a chromogenic agar according to the definition of EN ISO 11290 (Ottaviani Agosti formulation type) or forming part of a NF VALIDATION certified method, followed by a single reading or biochemical tests.

The complete protocols of the alternative method, including the confirmation steps, are shown in appendix 1.

1.3. [Reference method](#)

The reference method used was the one described in the standard EN ISO 11290-1/A1 (2004), "Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method".

The workflow of the method is set out in appendix 2.

1.4. [Application scope](#)

The scope of the method concerns a broad range of foods and environmental samples. In this document, categories are classified as below:

- ① meat products,
- ② dairy products,
- ③ seafood products,
- ④ vegetal products,
- ⑤ composite foods,
- ⑥ environmental samples.

2. [Method comparison study](#)

The results of the initial validation study and of the extension study are presented together for the parameters “sensitivity” and “relative level of detection”.

The parameters “inclusivity and exclusivity” and “practicability” were determined during the initial validation study and are not specific for a category.

2.1. [Sensitivity study](#)

The relative sensitivity is the ability of the alternative method to detect the analyte when it is detected by the reference method. The sensitivity study aims to determine the difference in sensitivity between the reference and the alternative method.

During the validation study, only the minimal incubation time of the broth of the alternative method was tested, namely: 22 hours for the general protocol and 18 hours for the protocol for environmental samples. Results were obtained using the Gene-UP Routine software version 1.0. Equivalency was accorded with the software version 1.1 and then 1.2.

All samples of the alternative method were confirmed by direct streaking of 10 µL of the enriched LPT broth on an ALOA Petri dish.

Typical colonies were confirmed by:

- the observation of the presence of typical colonies,
- the tests of the EN ISO 11290-1/A1 method including the purification step,
- a Rapidec L.mono test from an isolated colony,
- a Fast Rhamnose test from an isolated colony,
- an API *Listeria* gallery from an isolated colony.

In case of absence of typical colonies after a direct streaking from the enriched LPT broth, a subculture in 10 mL of Fraser broth was performed from 0.1 mL of the enriched LPT broth. This broth was incubated for 22 h at 37±1°C, then streaked on ALOA and PALCAM. Typical colonies were confirmed by the tests described above. For samples found negative with the Gene-UP *Listeria monocytogenes* test, a subculture of 0.1 ml of the LPT broth was performed into 10 mL of Fraser broth incubated then for 48 hours and then streaked onto an ALOA Petri dish to apply the extended confirmation protocol of the ISO 16140-2 standard for negative samples.

A storage of the DNA extracts for 72 h at 5±3°C was performed for all samples.

A storage of the enriched LPT broth for 72 h at 5±3°C was also performed for positive and discordant results. Results were confirmed by streaking on ALOA agar media, followed by a rapid test. In case of discordance in the confirmation result with the results obtained in first analysis, all kinds of confirmation of the alternative method were applied.

2.1.1. [Number and nature of samples](#)

The sensitivity study for all categories concerned 508 samples.

Samples, categories and types are presented in table 1.

Table 1 : Number and nature of samples analyzed for the initial validation (° : positive by any method)

Category	Type	Number of negative results	Number of positive results [°]	Total
Meat products ①	a Raw products (including deep-frozen, fresh, seasoned)	28	13	41
	b Ready-to-eat and processed meat products	14	11	25
	c Fermented or dried meat products (raw and cooked)	12	8	20
	Total	54	32	86
Dairy products ②	a Raw milk cheese	23	12	35
	b Other raw milk products	10	10	20
	c Heat-processed milk and dairy products	30	11	41
	Total	63	33	96
Seafood products ③	a Raw products	12	15	27
	b Smoked, marinated products	9	12	21
	c Processed products	12	8	20
	Total	33	35	68
Vegetal products ④	a Raw vegetal products	15	10	25
	b Ready-to-eat and ready-to-cook raw vegetal products, precooked vegetal products	15	9	24
	c Processed vegetal products	12	12	24
	Total	42	31	73
Composite foods ⑤	a Ready-to-eat foods	13	11	24
	b Ready-to-reheat foods	10	12	22
	c Pastries, egg products	12	10	22
	Total	35	33	68
Environmental samples (general and specific protocol) ⑥	a Process waters (general protocol)	10	15	25
	b Dusts and residues (general protocol)	14	7	21
	c Sponges and swabs (specific protocol)	41	30	71
	Total	65	52	117
Total all categories		292	216	508

2.1.2. [Artificial contamination of samples](#)

Artificial contaminations were performed using the seeding protocol mentioned in the standard ISO 16140-2: 2016: 138 samples were artificially contaminated.

The contamination levels varied between 0.2 and 7.0 CFU/25 g. No more than six positive results were obtained using the same strain.

Considering all the categories of the application scope, 216 samples gave a positive result by at least one of the method and 47.9 % of them were naturally contaminated.

The detail of the artificial contaminations is in appendix 3.

2.1.3. [Results](#)

Raw data are shown in appendix 4a.

Table 2 shows the results of the sensitivity study for the categories of the initial validation, for the categories of the extension study and for all categories.

Table 2 : results of the sensitivity study for both methods for the categories of the initial validation and of the extension study (PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumptive positive before confirmation, A+/R+ : confirmed positive, A-/R- negative immediately and negative after confirmation of the presumptive positive)

Category	Response	Reference method ^(*) positive (R+)	Reference method ^(*) negative (R-)
Meat products	Alternative method positive (A+)	PA= 25	PD= 4
	Alternative method negative (A-)	ND= 3 including 0 PPND	NA= 54 including 1 PPNA
Dairy products	Alternative method positive (A+)	PA= 23	PD= 5
	Alternative method negative (A-)	ND= 5 including 1 PPND	NA= 63 including 0 PPNA
Seafood products	Alternative method positive (A+)	PA= 20	PD= 7
	Alternative method negative (A-)	ND= 8 including 0 PPND	NA= 33 including 1 PPNA
Vegetal products	Alternative method positive (A+)	PA= 24	PD= 3
	Alternative method negative (A-)	ND= 4 including 0 PPND	NA= 42 including 1 PPNA
Composite foods	Alternative method positive (A+)	PA= 19	PD= 6
	Alternative method negative (A-)	ND= 8 including 0 PPND	NA= 35 including 4 PPNA
Environmental samples (specific protocol)	Alternative method positive (A+)	PA= 27	PD= 1
	Alternative method negative (A-)	ND= 2 including 0 PPND	NA= 41 including 2 PPNA
Environmental samples (general and specific protocol)	Alternative method positive (A+)	PA= 37	PD= 9
	Alternative method negative (A-)	ND= 6 including 0 PPND	NA= 65 including 3 PPNA
All categories	Alternative method positive (A+)	PA= 148	PD= 34
	Alternative method negative (A-)	ND= 34 including 1 PPND	NA= 292 including 10 PPNA

Comments:

- 1) For samples GL5, GL12, GL21 and GL23, the result of the confirmation using a RAPIDEC L. mono test was *Listeria ivanovii* whereas all other confirmations indicated the presence of *Listeria monocytogenes*. This result was probably due to a low quantity of biomass for the realization of the test. A new test after a streaking of a typical colony on an ALOA agar media gave the result *Listeria monocytogenes*.
- 2) The amplification curves and melting peaks of some samples are presented in appendices 5B (initial validation study) and 5C (extension study). The samples which are concerned present:
 - an early CP without MP: a CP is detected by the software, but not visible on the curves. This interpretation has no consequence on the final result which remains negative (no MP).
 - no CP but a MP: no CP and no MP are visible on the curves but a MP is nevertheless detected. This interpretation led to false positive results.All the DNA lysates from these samples were re-analyzed. They became negative (CP and MP equal to 0.00).
- 3) One inhibition was observed with sample GL270 (noodles chicken vegetables). The workflow to remove the inhibition has been applied successfully.

2.1.4. [Calculation and interpretation for sensitivity](#)

All results were used to calculate the sensitivity for the alternative method and the reference method and the relative sensitivity (cf. table 3).

Table 3 : values in % of sensitivity for the two methods, relative trueness and false positive ratio for the alternative method (PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumptive positive before confirmation, SE_{alt} : sensitivity for the alternative method, SE_{ref} : sensitivity for the reference method, RT: relative trueness, FPR: false positive ratio for the alternative method)

Category	Type	PA	NA	ND	PD	N	PPND	PPNA	SE_{alt}	SE_{ref}	RT	FPR
Meat products ①	a	9	28	2	2	41	0	1	84,6%	84,6%	90,2%	3,6%
	b	11	14	0	0	25	0	0	100,0%	100,0%	100,0%	0,0%
	c	5	12	1	2	20	0	0	87,5%	75,0%	85,0%	0,0%
	Total	25	54	3	4	86	0	1	90,6%	87,5%	91,9%	1,9%
Dairy products ②	a	10	23	1	1	35	0	0	91,7%	91,7%	94,3%	0,0%
	b	5	10	1	4	20	0	0	90,0%	60,0%	75,0%	0,0%
	c	8	30	3	0	41	1	0	72,7%	100,0%	92,7%	3,3%
	Total	23	63	5	5	96	1	0	84,8%	84,8%	89,6%	1,6%
Seafood products ③	a	9	12	1	5	27	0	0	93,3%	66,7%	77,8%	0,0%
	b	6	9	5	1	21	0	0	58,3%	91,7%	71,4%	0,0%
	c	5	12	2	1	20	0	1	75,0%	87,5%	85,0%	8,3%
	Total	20	33	8	7	68	0	1	77,1%	80,0%	77,9%	3,0%
Vegetal products ④	a	7	15	2	1	25	0	1	80,0%	90,0%	88,0%	6,7%
	b	8	15	0	1	24	0	0	100,0%	88,9%	95,8%	0,0%
	c	9	12	2	1	24	0	0	83,3%	91,7%	87,5%	0,0%
	Total	24	42	4	3	73	0	1	87,1%	90,3%	90,4%	2,4%
Composite foods ⑤	a	7	13	2	2	24	0	2	81,8%	81,8%	83,3%	15,4%
	b	7	10	2	3	22	0	2	83,3%	75,0%	77,3%	20,0%
	c	5	12	4	1	22	0	0	60,0%	90,0%	77,3%	0,0%
	Total	19	35	8	6	68	0	4	75,8%	81,8%	79,4%	11,4%
Environmental samples (general and specific protocol) ⑥	a	8	10	3	4	25	0	1	80,0%	73,3%	72,0%	10,0%
	b	2	14	1	4	21	0	0	85,7%	42,9%	76,2%	0,0%
	c	27	41	2	1	71	0	2	93,3%	96,7%	95,8%	4,9%
	Total	37	65	6	9	117	0	3	88,5%	82,7%	87,2%	4,6%
All categories	Total	148	292	34	34	508	1	10	84,3%	84,3%	86,6%	3,8%

2.1.5. Analysis of discordant results

Discordant results are examined according to the standard ISO 16140-2: 2016.

- **Positive deviations :**

A positive result is obtained by the alternative method whereas a negative result is obtained by the reference method. Due to the difference of sampling between both methods, no cell of *L. monocytogenes* may have been taken in the sampling for the reference method.

Positive deviations are listed in table 4.

Table 4 : Summary of the positive deviations

Sample number	Category	Product	Gene-UP result		
			CP	MP	Result
GL19	Meat products	Sausage with garlic	32.0	64.7	+
GL141		Raw chicken scallop	25.0	65.2	
GL159		Tournedos raw beef	24.0	64.9	
GL163		Ham without rind	24.9	64.9	
GL124	Dairy products	Butter (raw milk)	23.7	65.1	
GL125		Sweet churned butter (raw milk)	25.7	65.3	
GL128		Non fat fermented ribot milk	23.8	65.0	
GL130		Micro-filtered semi-skimmed milk	25.9	65.1	
GL167		Emmental (raw milk cheese)	26.6	64.4	
GL368	Seafoods	Fricassee	0	65.5	
GL378		Monkfish fillet	29.4	64.9	
GL383		Plaice fillet	25.5	64.9	
GL388		Smoked trout offcuts	23.8	64.7	
GL402		Trout fillet	30.6	65.1	
GL406		Swordfish	24.9	65.1	
GL408		Monkfish fillet	24.6	64.7	
GL201	Vegetables	Pre-cooked cauliflower	26.8	65	
GL349		Ratatouille	23.3	65	
GL358		Cherry tomatoes	23.7	62.1	
GL259	Composite foods	Mirabelles pie	27.5	65.4	
GL266		Pizza 4 cheese	23.1	64.7	
GL294		Chicken burger	26.0	62.1	
GL302		Ground beef sandwich	23.8	62.1	
GL319		Salad pineapple, carrot, surimi	26.3	62.2	
GL322		Poultry tabouleh	24.7	65	
GL498	Environment	Dust 6	29.5	65	
GL500		Dust 8	22.6	64.4	
GL513		Swab 20	21.9	64.5	
GL567		Process water 36	17.5	64.8	
GL568		Process water 37	18.5	65.7	
GL570		Process water 39	19.3	64.9	
GL573		Process water 42	27.3	62.5	
GL575		Dust 24	24.3	62.0	
GL589		Dust 26	18.8	61.6	

- **Negative deviations :**

A positive result is obtained by the reference method whereas a negative result is obtained by the alternative method.

Due to the difference of sampling between both methods, no cell of *L. monocytogenes* may have been present in the sampling of the alternative method.

Negative deviations are listed in table 5.

Table 5 : Summary of the negative deviations

Sample number	Category	Product	Gene-UP result			Confirmation
			CP	MP	Result	
GL25	Meat products	Raw boneless leg of lamb	0	0	-	-
GL149		Raw sliced horse meat	0	0	-	-
GL161		Speck	0	0	-	-
GL126	Dairy products	Sweet churned butter (raw milk)	0	0	-	-
GL129		Pasteurized semi-skimmed milk	0	0	-	-
GL132		Raw milk	0	51.3	+	-
GL171		Raw goat milk cheese	0	0	-	-
GL173		Pasteurized ewe milk cheese	0	0	-	-
GL366	Seafoods	Smoked salmon	0	0	-	-
GL371		Smoked salmon	0	0	-	-
GL375		Marinated tuna carpaccio	0	0	-	-
GL382		Cod fillet	0	0	-	-
GL387		Smoked trout	0	0	-	-
GL390		Shrimp tails marinated with garlic and parsley	0	0	-	-
GL393		Tuna à la catalane	0	0	-	-
GL394		Salmon rillettes	0	0	-	-
GL199	Vegetables	Frozen porcini mushroom	0	0	-	-
GL204		Basil	0	0	-	-
GL 346		Pesto with fresh basil	0	0	-	-
GL 353		Carrot purée	0	0	-	-
GL255	Composite foods	Apricots pie	0	0	-	-
GL257		Apple pie	0	0	-	-
GL258		Flan	0	0	-	-
GL262		Chicken salad, raw vegetables	0	0	-	-
GL296		Quiche lorraine	0	0	-	-
GL297		Tart with tomatoes and chorizo	0	0	-	+
GL298		Chocolate cake	0	0	-	-
GL320		Prawns salad with mandarins	0	0	-	-
GL462	Environment	Sponge 5	0	0	-	-
GL467		Process water 4	0	0	-	-
GL499		Dust 7	0	0	-	-
GL525		Sponge 8	0	0	-	-
GL569		Process water 38	0	0	-	-
GL574		Process water 43	0	0	-	-

Table 6 show the difference between negative deviations and positive deviations and the acceptability limits.

Table 6: acceptability limits

Category	Type	ND	PD	(ND-PD)	Acceptability limit (AL)	Observation
Meat products ①	a	2	2	/	/	(ND-PD) ≤ AL :
	b	0	0			
	c	1	2			
	Total	3	4	-1	3	
Dairy products ②	a	1	1	/	/	
	b	1	4			
	c	3	0			
	Total	5	5	0	3	
Seafood products ③	a	1	5	/	/	
	b	5	1			
	c	2	1			
	Total	8	7	1	3	
Vegetal products ④	a	2	1	/	/	
	b	0	1			
	c	2	1			
	Total	4	3	1	3	
Composite foods ⑤	a	2	2	/	/	
	b	2	3			
	c	4	1			
	Total	8	6	2	3	
Environmental samples (general and specific protocol) ⑥	a	3	4	/	/	
	b	1	4			
	c	2	1			
	Total	6	9	-3	3	
All categories	Total	34	34	0	6	

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

2.1.6. [Comments on confirmations](#)

- **Extended confirmation procedure (Gene-UP and ISO 16140-2 workflows)**

Two samples were confirmed positive after the application of the extended confirmation procedure: GL228 and GL356 (vegetal products).

- **False positive results**

Eleven samples found positive with the Gene-UP LMO method were not confirmed:

Nine samples showed an absence of CP but the presence of a MP. These samples may correspond to a false detection by the software. The curves obtained for such samples are displayed in appendices 4B and 4C. For one sample (GL206, chive), a clear positive signal was obtained but it was not possible to confirm the presence of *L. monocytogenes* in the LPT broth.

All the DNA lysates from these eleven samples we re-analyzed. They became negative (CP and MP equal to 0.00), except samples GL48 and GL206 which remained positive.

- **False negative results**

For four samples: GL297 and GL318 (composite foods) and GL228 and GL356 (vegetal products), a negative result is obtained by the alternative method. However the confirmation protocols allowed finding typical colonies which were confirmed as *Listeria monocytogenes*.

For these samples, it is probable that the enrichment did not allow to reach the threshold of the GENE-UP method.

It's important to note that the result of the reference method for samples GL228, GL318 and GL356 showed also an absence of *Listeria monocytogenes*.

2.1.7. Study of storage of the DNA extracts

A stability study of the DNA lysates stored at $5\pm 3^{\circ}\text{C}$ for 72 hours was performed on all samples. After storage, the lysates were re-analyzed.

Eight modifications appeared between the analysis just after the lysis step and after three days of storage: they concern false positive results that became negative after three days of storage for 7 samples: GL425 (seafood), GL275, GL276, GL283 (composite foods), GL481, GL545 and GL562 (environment).

Sample GL356 (vegetables), found positive after storage of the DNA, was confirmed positive after the extended confirmation procedure only (positive deviation).

2.1.8. Study of storage of the enriched LPT broths

A stability study of the enriched LPT broths stored at $5\pm 3^{\circ}\text{C}$ for 72 hours was performed on all positive and discordant samples. After storage, the broths were re-analyzed and confirmed with an isolation on an ALOA agar media (results in appendix 4a and analysis of discordant results in table 7).

Seven modifications appeared: six false positive results became negative after three days of storage and one sample, found positive after enrichment and after storage of the LPT broth for 72 hours at $2-8^{\circ}\text{C}$, was unable to be confirmed after the storage of the LPT broth, even after a subculture of the LPT broth in Fraser broth.

Table 7: acceptability limits after three days of storage at 5±3°C of the enrichment broth

Category	Type	ND	PD	(ND-PD)	Acceptability limit (AL)	Observation
Meat products ①	a	2	2	/	/	(ND-PD) ≤ AL
	b	0	0			
	c	1	2			
	Total	3	4	-1	3	
Dairy products ②	a	1	1	/	/	
	b	1	4			
	c	3	0			
	Total	5	5	0	3	
Seafood products ③	a	1	5	/	/	
	b	5	1			
	c	2	1			
	Total	8	7	1	3	
Vegetal products ④	a	2	1	/	/	
	b	0	1			
	c	2	1			
	Total	4	3	1	3	
Composite foods ⑤	a	2	2	/	/	
	b	2	3			
	c	4	1			
	Total	8	6	2	3	
Environmental samples (general and specific protocol) ⑥	a	3	4	/	/	
	b	1	4			
	c	2	1			
	Total	6	9	-3	3	
All categories	Total	34	34	0	6	

The observed values (ND – PD) are below the acceptability limit for each category and for all categories. The alternative method produces results comparable to the reference method. These results did not modify the conclusion for the conservation of the broths.

2.2. [Relative level of detection study](#)

The level of detection is the measured analyte concentration, obtained by a given measurement procedure, for which the probability of detection is x, e.g. LOD50 is the level of detection for which 50 % of tests give a positive result.

The relative level of detection (RLOD) is defined as the level of detection at P = 0.50 (LOD50) of the alternative (proprietary) method divided by the level of detection at P = 0.50 (LOD50) of the reference method.

2.2.1. [Experimental design](#)

Seven matrix-strain couples were studied in parallel by both methods. For each category of the scope, one relevant type of food product is selected. Three levels of contamination per type were prepared consisting of a negative control level, a low level, and a higher level. Only one strain of the target analyte is used to contaminate the low and the high level.

The negative control level shall not produce positive results. Five replicates are tested for this level.

The low level shall be the theoretical detection level, it has been contaminated at 0.7 - 1 CFU per test portion to obtain fractional recovery results. Twenty replicates are tested for this level.

The higher level shall be just above the theoretical detection level, it has been contaminated at 2 - 3 CFU per test portion. Five replicates are tested for this level.

Food products were contaminated using the seeding protocol. Bulk contaminations were performed on the matrices for the different levels of contamination, then the matrices were stored at 5±3°C for two days before analysis. Samples were then analyzed by the reference and the alternative method.

For the alternative method, only the minimal incubation time of the broth of the alternative method was tested, namely 22 hours for the general protocol and 18 hours for the specific protocol. Results were obtained using the Gene-UP Routine software version 1.0.

Simultaneously, a total viable count was performed on a portion of non-contaminated matrix to estimate the concentration of mesophilic aerobic flora. A detection of *Listeria monocytogenes* using the reference method was also performed to check the absence of the target analyte in the matrix.

Table 8 details the two couples matrix-strain tested.

Table 8 : couples matrix-strain used for the determination of the RLOD of the method

Category	Matrix type	Strain	Code	Strain origin
Meat products	Pork rillettes	<i>Listeria monocytogenes</i> 1/2c	LIS.4.33	Minced meat
Dairy products	Raw milk	<i>Listeria monocytogenes</i> 1/2b	LIS.4.32	Raw milk
Seafood products	Salmon offcuts	<i>Listeria monocytogenes</i> 4b	LIS.4.50	Swab on salmon
Vegetal products	Mix of precooked vegetables	<i>Listeria monocytogenes</i> 1/2a	LIS.4.10	Salad
Composite foods	Mixed salad	<i>Listeria monocytogenes</i> 1/2c	LIS 4.35	Vegetables sandwich
Environmental samples (surface samples)	Swab on a surface	<i>Listeria monocytogenes</i> 3a	LIS.4.44	Surface control
Environmental samples (except surface samples)	Process water	<i>Listeria monocytogenes</i> 1/2a	LIS.4.16	Surface control drainage point

2.2.2. Results and calculation of the RLODs

Raw results are shown in appendix 5.

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>. Values of the RLODs are presented in table 9.

Table 9 : RLODs values for the six 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, $b=\ln(\text{RLOD})$: 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 $H_0: b=0$, p-value: p-value of the z-Test)

Category	RLOD	RLODL	RLODU	$b=\ln(\text{RLOD})$	$sd(b)$	z-Test statistic	p-value	Acceptability limit
Meat products	0.678	0.275	1.668	-0.389	0.450	0.864	1.612	2.5
Dairy products	1.313	0.527	3.271	0.272	0.457	0.596	0.551	
Seafood products	1.533	0.686	3.424	0.427	0.402	1.063	0.288	
Vegetal products	0.565	0.241	1.322	-0.572	0.426	1.344	1.821	
Composite foods	0.667	0.320	1.390	-0.404	0.367	1.103	1.730	
Environmental samples (surface samples)	0.756	0.291	1.969	-0.279	0.478	0.584	1.441	
Environmental samples (except surface samples)	1.146	0.498	2.636	0.136	0.417	0.327	0.744	
Combined	0.890	0.661	1.198	-0.117	0.149	0.786	1.568	

2.2.3. Interpretation and conclusion

The RLODs values are below the acceptability limit set at 2.5, meaning that, as stated in ISO 16140-2: 2016, the maximum increase in LOD of the alternative versus the reference method is not considered as relevant in consideration of the fitness for purpose of the method.

In conclusion, alternative and reference methods show similar LODs values for the detection of *Listeria monocytogenes* in the categories tested.

2.3. Inclusivity and exclusivity study

Fifty target strains and thirty non-target strains were analyzed by the alternative method.

2.3.1. Test protocols

For target strains, 225 mL of LPT broth were inoculated with 10 to 50 cells of *Listeria monocytogenes*. The complete protocol of the alternative method was then applied after an incubation at the minimum enrichment time of the alternative method (22 h). Results were obtained using the Gene-UP Routine software version 1.0.

Positive results were confirmed by streaking of the enriched broth on ALOA agar media, incubated for 24 to 48 h at $37\pm 1^\circ\text{C}$.

For non-target strains, cells were cultivated first overnight in a non-selective broth (BHI) at 10^5 CFU/mL. The protocol of the alternative method was then applied.

2.3.2. Results

Results are shown in appendix 6.

All target strains were detected by the alternative method.

None of the non-target strains showed a cross reaction with the alternative method.

2.3.3. Conclusion

The inclusivity and the exclusivity of the alternative method are satisfactory.

All target strains were detected by the alternative method.

None of the non-target strains showed a cross-reaction with the alternative method.

The inclusivity and the exclusivity of the alternative method are satisfactory.

2.4. Practicability

The practicability of the alternative method was informed according to the criteria defined by the Technical Committee.

1. Storage conditions, shelf-life and modalities of utilization after first use

The GENE-UP kits are stored at room temperature (15-25°C) and have not to be refrigerated.

After opening a kit, the pouches have to be correctly sealed and undamaged. If not, they have not to be used.

Once pouches are opened, freeze-dried pellets should be reconstituted and used within 30 days. Freeze-dried pellets should be stored in original sealed pouch (with lab adhesive or bag clip).

Once freeze-dried pellets are reconstituted, testing on the GENE-UP Thermocycler should be initiated as soon as possible. Storage conditions for vials are the following: 2 hours at ambient temperature, 2 days at 2 – 8°C, 8 days at -20°C.

2. Time-to-result

Negative results are obtained in one day.

Positive results are obtained in two to three days.

3. Common step with the reference method

The alternative method has no common step with the reference method.

3. [Interlaboratory study](#)

A first session was realized in April 2016. Its results were presented at the Technical Board meetings of June and September 2016. After removal of 6 data sets as explained below, less than 10 data sets were available for the statistical interpretation of the results.

An additional interlaboratory study, including 4 collaborators was realized in September 2016 in the same conditions.

The two studies were combined for the final interpretation.

3.1. [Interlaboratory study organization](#)

3.1.1. [Collaborators](#)

The first interlaboratory study was realized by the expert laboratory and fifteen collaborators coming from fourteen different organizations.

The second interlaboratory study was realized by the expert laboratory and four collaborators coming from four different organizations.

Each laboratory received the instructions relative to the organization of the study a week before its beginning.

3.1.2. [Matrix and strain of *Listeria monocytogenes*](#)

A full-cream goat milk cottage cheese was used as test matrix for the two studies. Its ingredients are the following: pasteurized full-cream goat milk, milk enzymes, rennet. Its fat content is 8.4%.

It was contaminated by a strain of *Listeria monocytogenes* 1/2 b, isolated from a raw milk (ISHA code: LIS.4.67).

The absence of *Listeria monocytogenes* in the matrix before contamination was checked using the reference method.

3.1.3. [Stability of the strain in the test matrix](#)

The stability of the strain in the matrix was evaluated for 4 days at $5\pm 3^{\circ}\text{C}$ before the first interlaboratory study. Samples for enumeration were contaminated at a level close to 100 CFU/g. Samples for detection were inoculated at a level from 1 to 3 CFU/25 g. Results of the analyses are presented in table 10. No significant variation of the *L. monocytogenes* count was observed until Day 3.

Table 20 : determination of the stability of the strain of *L. monocytogenes* inoculated in the test matrix

Day	Enumeration CFU/g	Alternative method	Reference method
D0	160	Presence in 25 g	Presence in 25 g
D1	200	Presence in 25 g	Presence in 25 g
D2	140	Presence in 25 g	Presence in 25 g
D3	210	Presence in 25 g	Presence in 25 g

3.1.4. [Preparation and contamination of the sample](#)

The matrix was inoculated with the target strain suspension to obtain 3 contamination levels:

- L0 : 0 cell in 25 g,
- L1 : 0.7 – 1 cells in 25 g,
- L2 : 10 cells in 25 g.

Twenty-five grams of matrix were distributed in sterile bags. Each bag was individually contaminated and homogenized. Eight samples per level, per collaborator and per method were prepared. Each laboratory

received 48 samples to analyze, one sample to perform the total viable count (TVC) and one water sample containing a temperature probe.

The results of the TVC, the target levels and the real levels of contamination are presented in table 11.

Table 11 : target level, real level and endogenous flora of the matrix

Interlaboratory study	TVC (CFU/g)	Target level (cells/25 g)	Real level (cells/25 g)	Confidence interval
April 2016	3,0.10 ⁶	0	/	/
		0.7 – 1	1.9	[1.5 ; 2.4]
		10	9.2	[7.2 ; 11.4]
September 2016	9,8.10 ⁴	0	/	/
		0.7 – 1	1.2	[0.8 ; 1.6]
		10	10.8	[8.7 ; 12.9]

3.1.5. Labelling of the samples

Labelling of the bags was realized as follows:

- a code to identify the laboratory: from A to O for the first study, and from P to S for the second study,
- and a code to identify each sample, only known by the expert laboratory.

The samples and the temperature control vials (water sample with a temperature probe) were stored at 5±3°C before shipping.

3.1.6. Shipping and receipt of the samples, analyses by the collaborators

The samples were shipped in a coolbox.

The control temperature was recorded upon receipt of the package and the temperature probe sent to the expert laboratory.

The samples had to be analyzed one or two days after the shipping.

The expert laboratory concurrently analyzed a set of samples under the same conditions with both methods.

For the first study, it is important to note that two series of analyses were realized by the collaborators for the detection with the alternative method:

- one at the following of the lysis step for the regular workflow of the method but with a detection kit which should not have been used after an error in the delivery of the kits,
- the other after storage of the DNA a few days at -20°C with a kit capable of being used.

For all collaborators except one, samples which were presumed positive not confirmed or inhibited with the first detection kit were found negative with the second detection kit.

Only collaborator C obtained four samples inhibited with the second detection kit, when it had only one sample presumed positive not confirmed with the first detection kit. This laboratory did not preserve lysates and was not thus able to re-test the inhibited samples. That's why the results of this collaborator are not kept in the statistical analysis, because no answer is obtained for four samples. Results of this collaborator are presented only in appendices.

Only analyses performed with the second detection kit (the valid kit) were taken into account for the compilation of the data.

3.2. Results

3.2.1. Temperature and state of the samples at receipt

The temperature readings upon reception and the state of the samples are shown in table 12.

Table 12 : temperature and state of the samples at reception (/: data unable to be gathered)

Collaborator	Date and time of receipt	Temperature (°C) determined by the collaborator	Mean temperature (°C) during the shipping given by the probe	State of the samples	Date of analysis
A	04/26/2016 at 11:30	3.8	2.4	Correct	04/26/2016
B	04/26/2016 at 12:30	2.8	/	Correct	04/27/2016
C	04/26/2016 at 11:45	3.6	2.0	Correct	04/26/2016
D	04/26/2016 at 11:35	5.2	2.5	Correct	04/27/2016
E	04/26/2016 at 10:05	2.6	/	Correct	04/26/2016
F	04/26/2016 at 12:30	7.3	/	Correct	04/27/2016
G	04/26/2016 at 11:00	6.6	4.8	Correct	04/26/2016
H	04/27/2016 at 10:30	3.0	2.9	Correct	04/27/2016
I	04/27/2016 at 10:00	6.5	2.0	Correct	04/28/2016
J	04/26/2016 at 09:35	4.0	3.9	Correct	04/26/2016
K	04/26/2016 at 07:30	2.9	3.7	2 samples leaky	/
L	04/26/2016 at 11:15	4.0	3.5	Correct	04/26/2016
M	04/26/2016 at 10:00	2.9	4.9	Correct	04/26/2016
N	04/26/2016 at 10:20	2.8	1.4	Correct	04/26/2016
O	04/26/2016 at 11:35	5.3	2.5	Correct	04/27/2016
P	06/09/2016 at 11:00	7.8	6.1	Correct	07/09/2016
Q	06/09/2016 at 10:30	2.6	1.9	Correct	06/09/2016
R	06/09/2016 at 11:00	5.0	2.5	Correct	06/09/2016
S	06/09/2016 at 12:00	1.5	-0.8	Correct	07/09/2016

- **First interlaboratory study**

Only laboratory K didn't receive the sample in adequate conditions: two samples were leaky. This collaborator did not thus realize the analyses. All other laboratories received the samples in appropriate conditions. Temperatures during the shipping and upon receipt were correct for all laboratories.

Collaborator I realized the analyses (alternative and reference method) the 28th of April 2016, so three days after shipping, because of a late delivery of the reagents of the methods.

The stability of the strain in the matrix was tested for four days (from D0 to D3) by the expert laboratory and was correct. Moreover the samples of this collaborator were kept cold after reception. The request from the expert laboratory to keep the results of this collaborator in the final data was not accepted by the Technical Board of September 2016.

The results of collaborator I are thus excluded from the final set of data.

The results of collaborator C were already excluded from the statistical analysis of the data because of incomplete results (see § 3.1.6.1).

So fourteen collaborators realized the analyses and the results of only twelve can be taken into account.

- **Second interlaboratory study**

The temperature is found slightly inferior to 0°C for a part of the shipping of the collaborator S package. This may be due to a bad positioning of the thermal probe vial after the packaging, which has probably moved beside a -20°C ice pack. Collaborator S didn't report that the samples were frozen at reception.

Temperatures during the shipping for the other collaborators were correct. Temperatures at reception for all collaborators were correct.

3.2.2. [Expert laboratory results](#)

The results obtained by the expert laboratory are summarized in table 13.

Raw results are presented in appendices 7 and 9.

Table 13 : positive results obtained by expert laboratory by both methods

Interlaboratory study	Contamination level	Alternative method	Reference method (*)
April 2016	L0	0/8	0/8
	L1	3/8	1/8
	L2	8/8	8/8
September 2016	L0	0/8	0/8
	L1	7/8	6/8
	L2	8/8	8/8

The enumeration of the TVC by the expert laboratory gave the result of 4.7×10^8 CFU/g for the first interlaboratory study and 2.3×10^5 CFU/g for the second interlaboratory study.

3.2.3. [Collaborators results](#)

Raw results are presented in appendices 8 and 10.

3.2.3.1. [Mesophilic aerobic flora](#)

- **First interlaboratory study**

For the whole laboratories, the total viable count varied between 6.6×10^2 CFU/g and 4.8×10^8 CFU/g.

It is important to note that only two collaborators from the same laboratory found a TVC below 3.0×10^7 CFU/g. This laboratory indicated that the readings of the Petri dishes were performed by technicians not familiar with enumeration techniques which have probably counted only the colonies at the surface of the agar media for the first dilutions of the sample.

The TVCs observed by all other collaborators and by the expert laboratory are between 3.0×10^7 CFU/g and 4.8×10^8 CFU/g.

- **Second interlaboratory study**

For all laboratories, the total viable count varied between 2.0×10^3 CFU/g and 1.4×10^5 CFU/g.

3.2.3.2. Results of the reference method

Positive results of the collaborators for the reference method are presented in the table below.

Table 14 : positive results of the reference method for all laboratories

Collaborator	Contamination level		
	L0	L1	L2
A	0 / 8	8 / 8	8 / 8
B	0 / 8	8 / 8	8 / 8
D	0 / 8	8 / 8	8 / 8
E	0 / 8	8 / 8	8 / 8
F	0 / 8	7 / 8	8 / 8
G	0 / 8	8 / 8	8 / 8
H	2 / 8	2 / 8	8 / 8
I	0 / 8	2 / 8	8 / 8
J	0 / 8	4 / 8	8 / 8
L	0 / 8	8 / 8	8 / 8
M	0 / 8	8 / 8	8 / 8
N	0 / 8	8 / 8	8 / 8
O	0 / 8	8 / 8	8 / 8
P	0 / 8	6 / 8	8 / 8
Q	0 / 8	5 / 8	8 / 8
R	0 / 8	7 / 8	8 / 8
S	0 / 8	5 / 8	8 / 8
TOTAL	2 / 136	110 / 136	136 / 136

3.2.3.3. Results of the alternative method

Positive results of the collaborators for the alternative method are presented in the table below.

Table 15 : positive results of the alternative method for all laboratories (BC / AC: before / after confirmation)

Collaborator	Contamination level					
	L0		L1		L2	
	BC	AC	BC	AC	BC	AC
A	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
B	0 / 8	0 / 8	7 / 8	7 / 8	8 / 8	8 / 8
D	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
E	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
F	3 / 8	2 / 8	7 / 8	7 / 8	8 / 8	8 / 8
G	0 / 8	0 / 8	5 / 8	5 / 8	8 / 8	8 / 8
H	0 / 8	0 / 8	2 / 8	2 / 8	8 / 8	8 / 8
I	0 / 8	0 / 8	1 / 8	1 / 8	8 / 8	8 / 8
J	0 / 8	0 / 8	4 / 8	4 / 8	8 / 8	8 / 8
L	0 / 8	0 / 8	6 / 8	6 / 8	8 / 8	8 / 8
M	0 / 8	0 / 8	7 / 8	7 / 8	8 / 8	8 / 8
N	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
O	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
P	0 / 8	0 / 8	5 / 8	5 / 8	8 / 8	8 / 8
Q	1 / 8	0 / 8	7 / 8	7 / 8	8 / 8	8 / 8
R	0 / 8	0 / 8	4 / 8	4 / 8	8 / 8	8 / 8
S	0 / 8	0 / 8	4 / 8	4 / 8	8 / 8	8 / 8
TOTAL	4 / 136	2 / 136	99 / 136	99 / 136	136 / 136	136 / 136

3.2.4. Analysis of the results

3.2.4.1. Level 0

According to the specific requirements of the Technical Board linked to the standard ISO 16140-2: 2016, collaborators who obtained more than one positive result at level 0 (confirmed or not) per method must be excluded from the statistical analysis of the results.

This case happens, only during the first interlaboratory study, for:

- collaborator F: 1 presumptive positive result not confirmed and 2 confirmed positive results with the alternative method,
- collaborator H: 2 positive results obtained with the reference method.

3.2.4.2. Level 2

For the level 2, all results are consistent with those expected for all collaborators, namely a detection of all samples of the level by the two methods for the two interlaboratory studies.

3.2.4.3. Level 1

- **First interlaboratory study**

The results shown by the alternative method for all negative deviations observed at level 1 is: CT: 0,00 / MP: 0,00, so an absence of amplification and no detection of a melting peak.

For collaborators B, F and M, the presence of one non-detection at the level 1 could be due to the absence of *Listeria monocytogenes* in the sample analyzed because of the low inoculation level.

For collaborators who obtained a lot of negative results by any method at the level 1, an investigation was performed by the expert laboratory to try to explain deviations between the two methods.

Laboratory G prepared the initial suspensions for the alternative method with refrigerated LPT broths (not warmed to ambient temperature). This way to perform the analyses doesn't meet the requirements of the user guide and can delay the growth of *Listeria monocytogenes*. The proposal of the expert laboratory to exclude this collaborator from the statistical analysis was accepted by the Technical Board.

Laboratory L also used LPT broths not fully warmed to ambient temperature. Investigations showed that the LPT broths were at a 12°C when the initial suspensions were prepared. The Technical Board accepted the proposal of the Expert Laboratory to keep this collaborator for the statistical analysis.

For collaborators H, I and J, numbers of PD and ND were well balanced at level L1 between alternative method and reference method.

- **Second interlaboratory study**

The results shown by the alternative method for all negative deviations observed at level 1 were: CT: 0,00 / MP: 0,00, so an absence of amplification and no detection of a melting peak.

For collaborators P, Q and S, negative samples clearly came from an absence of *Listeria monocytogenes* in the samples as the confirmations were negative. Moreover, considering these three collaborators, positive and negative deviations were well balanced.

For collaborator R, four samples (3 / 8 / 15 / 16) were not detected by the alternative method despite the presence of *Listeria monocytogenes* in the broth.

It seems that the protocol of the alternative method has been correctly applied.

A second analysis of these samples using the lysates stored at -20°C gave three positive results and one negative result.

A summary of these deviations for this collaborator are presented in the table below:

Sample	Gene-UP result / first analysis			Gene-UP result / second analysis from the lysate stored at -20°C			Confirmation
	CP	MP	Result	CP	MP	Result	
3	0.00	0.00	-	27.16	66.69	+	Positive
8	0.00	0.00	-	24.93	66.28	+	Positive
15	0.00	0.00	-	0.00	0.00	-	Positive
16	0.00	0.00	-	25.36	66.26	+	Positive

For three samples out of four, a clear positive result is obtained for the re-test, which indicates the presence of the DNA target in the lysate. That's why, for this collaborator, a manipulation issue during the analysis is suspected, like an error of pipetting.

3.2.5. Conclusion

After having removed collaborators C, F, G, H, I and K from the results of the first interlaboratory study, nine sets of data were available for the statistical analysis.

As the standard ISO 16140-2: 2016 requires at least 10 valid data sets, the data from the second interlaboratory study was taken into account. For this second study, following the decision of the Technical Board of November 2016, the interpretation of the results was performed with the results of the first analysis of collaborator R.

3.2.6. Results kept for the statistical interpretation

Results kept are presented in tables below.

Table 16 : positive results of the reference method for laboratories kept for the statistical analysis

Interlaboratory study	Collaborator	Contamination level		
		L0	L1	L2
April	A	0 / 8	8 / 8	8 / 8
	B	0 / 8	8 / 8	8 / 8
	D	0 / 8	8 / 8	8 / 8
	E	0 / 8	8 / 8	8 / 8
	J	0 / 8	4 / 8	8 / 8
	L	0 / 8	8 / 8	8 / 8
	M	0 / 8	8 / 8	8 / 8
	N	0 / 8	8 / 8	8 / 8
	O	0 / 8	8 / 8	8 / 8
	TOTAL	0 / 72	68 / 72	72 / 72
September	P	0 / 8	6 / 8	8 / 8
	Q	0 / 8	5 / 8	8 / 8
	R	0 / 8	7 / 8	8 / 8
	S	0 / 8	5 / 8	8 / 8
	TOTAL	0 / 32	23 / 32	32 / 32
TOTAL		0 / 104	91 / 104	104 / 104

Table 17 : positive results of the alternative method for laboratories kept for the statistical analysis

Inter-laboratory study	Collaborator	Contamination level					
		L0		L1		L2	
		Before confirmation	After confirmation	Before confirmation	After confirmation	Before confirmation	After confirmation
April	A	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
	B	0 / 8	0 / 8	7 / 8	7 / 8	8 / 8	8 / 8
	D	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
	E	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
	J	0 / 8	0 / 8	4 / 8	4 / 8	8 / 8	8 / 8
	L	0 / 8	0 / 8	6 / 8	6 / 8	8 / 8	8 / 8
	M	0 / 8	0 / 8	7 / 8	7 / 8	8 / 8	8 / 8
	N	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
	O	0 / 8	0 / 8	8 / 8	8 / 8	8 / 8	8 / 8
	TOTAL	0 / 72	0 / 72	64 / 72	64 / 72	72 / 72	72 / 72
September	P	0 / 8	0 / 8	5 / 8	5 / 8	8 / 8	8 / 8
	Q	1 / 8	0 / 8	7 / 8	7 / 8	8 / 8	8 / 8
	R	0 / 8	0 / 8	4 / 8	4 / 8	8 / 8	8 / 8
	S	0 / 8	0 / 8	4 / 8	4 / 8	8 / 8	8 / 8
	TOTAL	1 / 32	0 / 32	20 / 32	20 / 32	32 / 32	32 / 32
TOTAL	1 / 104	0 / 104	84 / 104	84 / 104	104 / 104	104 / 104	

3.3. Interpretation of the results

3.3.1. Summary of the results

The global results are presented in the table below.

Table 18 : tests results for the two methods (PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumed positive before confirmation)

Level	Alternative method	Reference method	
		Reference method positive (RM+)	Reference method negative (RM-)
L0	Alternative method positive (AM+)	PA = 0	PD = 0
	Alternative method negative (AM-)	ND = 0 including 0 PPND	NA = 104 including 1 PPNA
L1	Alternative method positive (AM+)	PA = 74	PD = 10
	Alternative method negative (AM-)	ND = 17 including 0 PPND	NA = 3 including 0 PPNA
L2	Alternative method positive (AM+)	PA = 104	PD = 0
	Alternative method negative (AM-)	ND = 0 including 0 PPND	NA = 0 including 0 PPNA
L0+L1+L2	Alternative method positive (AM+)	PA = 178	PD = 10
	Alternative method negative (AM-)	ND = 17 including 0 PPND	NA = 107 including 1 PPNA

3.3.2. Calculation of sensitivities, relative accuracy and false positive ratio

Based on the three different data sets, the following parameters are calculated:

- Sensitivity for the alternative method: $SE_{alt} = \frac{(PA+PD)}{(PA+ND+PD)} \times 100\%$

- Sensitivity for the reference method: $SE_{ref} = \frac{(PA+ND)}{(PA+ND+PD)} \times 100\%$

- Relative accuracy: $AC = \frac{(PA+NA)}{N} \times 100\%$

- False positive ratio for the alternative method: $FP = \frac{(FP)}{NA} \times 100\%$

where N is the total number of samples (NA + PA + PD + ND) and FP is false positive results.

Results are presented in the table below.

Table 19 : values of sensitivities, relative accuracy and false positive ratio for the three data sets

Data set	Parameter			
	SE _{alt}	SE _{ref}	RT	FPR
Interlaboratory study 13 collaborators	91.7	95.1	91.3	0.9

3.3.3. Determination of the acceptability limit and conclusion

The difference between (ND – PD) for the level where fractional recovery was obtained (L1) is calculated. 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, (L1 or L2) for all laboratories;

Nx = number of samples tested at level x (L1 or L2) 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 (L1 or L2) for all laboratories;

Nx = number of samples tested at level x (L1 or L2) 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.

The AL is not met when the observed value is higher than the AL. When the AL is not met, 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 L1 only. The different parameters obtained by the calculation are detailed in the table below:

Table 20 : values obtained for the determination of the acceptability limit

Parameter	Value
N_x	104
$(p+)_{ref}$	0.875
$(p+)_{alt}$	0.808
$(ND-PD)_{max}$	9.17
$(ND-PD)$	7

The value (ND-PD) is inferior to the AL in all cases, so the requirements of the standard ISO 16140-2 : 2016 are fulfilled.

The performance of the alternative method and the reference method can be considered as equivalent.

3.3.4. Determination of the relative level of detection

This evaluation is performed according to Annex F of ISO 16140-2:2016 and using the excel spreadsheet as described in this standard.

As there is limited experience with the interpretation of this approach, the results are used only for information. Results are shown in the table below :

Table 21 : values obtained for the determination of the relative level of detection

Number of sets of data	RLOD	RLODL	RLODU	$b=\ln(RLOD)$	sd(b)	z-Test statistic	p-value
13 data sets	1,261	0,890	1,788	0,232	0,174	1,331	0,183

4. Conclusion

Overall, the study concerned 508 samples from six categories: meat products, dairy products, seafood products, vegetal products, composite foods and environmental samples.

The sensitivity of the alternative method was 84.3% and the sensitivity of the reference method was 84.3%. The observed values (ND – PD) were below to the acceptability limit for each category and for all categories.

The RLODs values were below the acceptability limits set at 2.5, meaning that, as stated in ISO 16140-2: 2016, the maximum increase in LOD of the alternative versus the reference method is not considered as relevant in consideration of the fitness for purpose of the method.

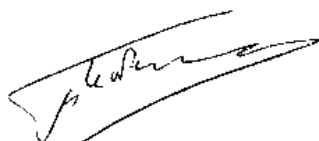
The GENE-UP *Listeria monocytogenes* method and the reference method show similar LODs values for the detection of *Listeria monocytogenes* in the categories tested.

The inclusivity and the exclusivity of the method showed that the method GENE-UP *Listeria monocytogenes* is specific and selective.

The practicability of the method highlighted a method quick and easy to apply.

The interlaboratory study, interpreted with the data of 13 collaborators, showed that the performance of the alternative method was equivalent to the performance of the reference method.

Considering all the categories of the application scope, namely a broad range of foods and environmental samples, the method GENE-UP *Listeria monocytogenes* produces results comparable to the reference method EN ISO 11290-1/A1 according to the standard ISO 16140-2: 2016.



Massy, March 17th, 2017,
François LE NESTOUR
Head of the Unit Innovation Biology

APPENDIX 1

ALTERNATIVE METHOD PROTOCOL

Protocol for a broad range of foods and environmental samples

Enrichment

Food sample and environmental sample except surface samples:

25 g sample + 225 LPT broth at room temperature in a blender bag.

Incubate for 22 – 28 h at 37±1°C.

Environmental surface samples:

Sponge or swipe + 100 mL LPT broth at room temperature in a blender bag.

Swab + 10 mL LPT broth at room temperature in a blender bag.

Incubate for 18 – 24 h at 37±1°C.

Lysis

Mix manually the content of the blender bag.

Transfer 20 µL of the enriched broth into a lysis tube.

Place the tube in a bead beater and run it for 5 minutes at a speed above 2 000 rpm.

Final setup for PCR

Reconstitute the PCR reagent according to the manufacturer's recommendations. A blue color shall be obtained.

Pipet 5 µL of the reagent in a PCR tube.

Using a 10 µL Biotix filter pipette tip, transfer 5 µL of the lysed sample (red color) into the PCR tube.

When sample is added to the PCR reagent, the solution turns purple.

Place a strip cap on each strip tube and seal it.

Spin in a plate centrifuge for 10 seconds.

The plate is now ready to be processed in the Gene-UP instrument and must be started within 15 minutes.

Results

Start the run according to the instructions of the manufacturer.

Read the results with the Gene-UP Routine software.

Confirmation

Confirm all positive results obtained with GENE-UP *Listeria monocytogenes*

Isolate 10 µL of the LPT broth on an ALOA agar plate.

Incubate for 24±3 h at 37°C.

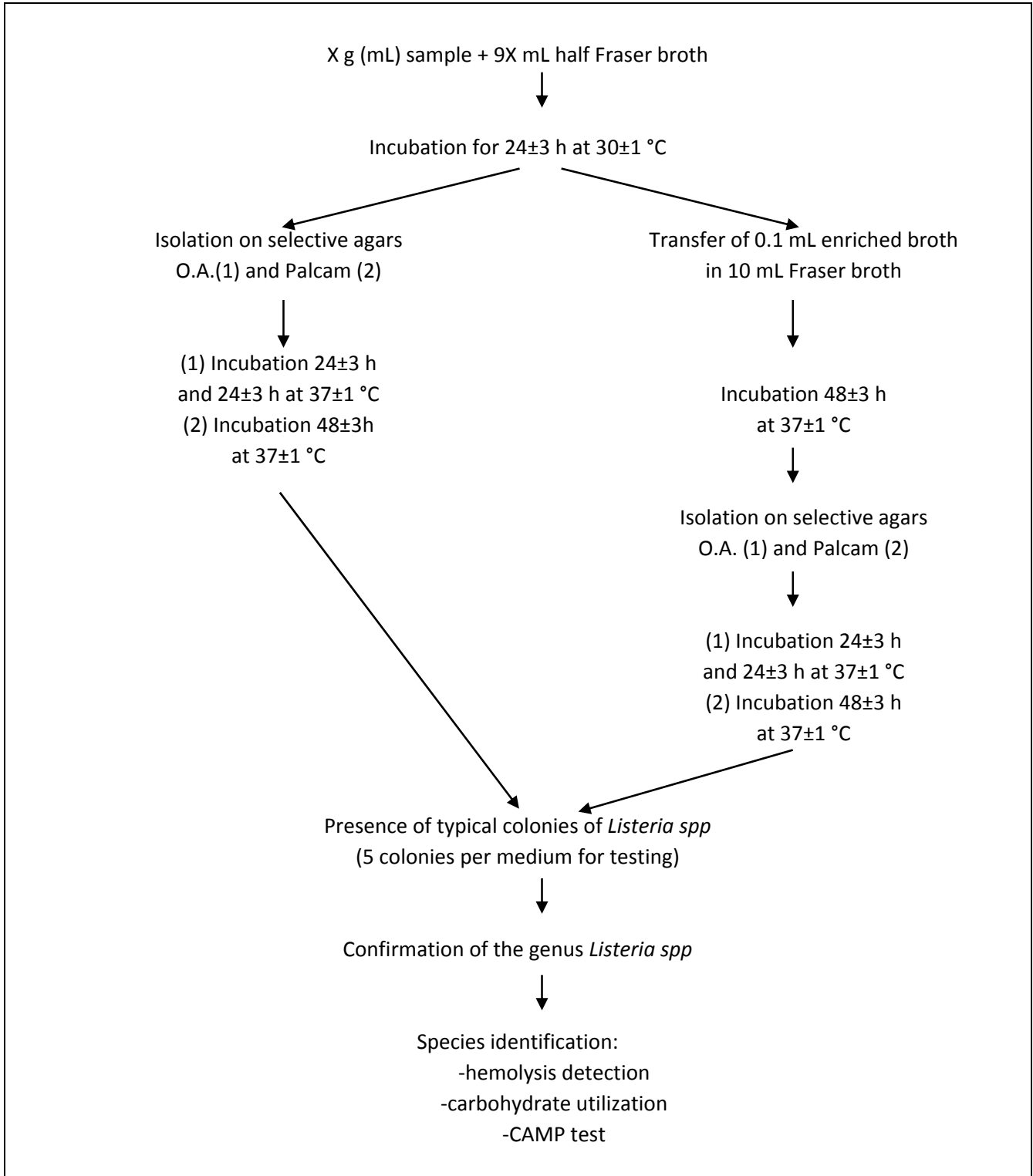
The plates can be read between 24 and 48 hours.

The presence of typical colonies confirms a positive result.

An API LIS strip or a RAPIDEC test can be performed directly from an isolated colony.

APPENDIX 2

REFERENCE METHOD PROTOCOL



APPENDIX 3

ARTIFICIAL CONTAMINATIONS

Category	Sample ID	Sample	Code strain	Strain	Origin	Protocol of seeding	Inoculation level (CFU/25g)	Global result
Meat products	GL29	Smoked lardons	LIS.4.20	<i>Listeria monocytogenes 1/2a</i>	sandwich bacon vegetables	48 h at 5±3°C	1.7	+
	GL30	Smoked bacon	LIS.4.27	<i>Listeria monocytogenes 1/2a</i>	Minced meat	48 h at 5±3°C	1.3	+
	GL31	Smoked cured ham	LIS.4.28	<i>Listeria monocytogenes 1/2b</i>	Leg of duck	48 h at 5±3°C	1.0	-
	GL32	Raw Montbéliard sausage	LIS.4.30	<i>Listeria monocytogenes 1/2b</i>	Raw turkey	48 h at 5±3°C	0.5	-
	GL159	Raw tournedos (beef)	LIS.4.11	<i>Listeria monocytogenes 1/2a</i>	Chicken with curry	48 h at 5±3°C	1.6	+
	GL160	Raw turkey filet mignon	LIS.4.11	<i>Listeria monocytogenes 1/2a</i>	Chicken with curry	48 h at 5±3°C	1.6	+
	GL161	Speck	LIS.4.11	<i>Listeria monocytogenes 1/2a</i>	Chicken with curry	48 h at 5±3°C	1.6	+
	GL162	Smoked salami	LIS.4.26	<i>Listeria monocytogenes 1/2a</i>	Ham	48 h at 5±3°C	2.8	-
	GL163	ham without rind	LIS.4.26	<i>Listeria monocytogenes 1/2a</i>	Ham	48 h at 5±3°C	2.8	+
GL164	Ham with rind	LIS.4.26	<i>Listeria monocytogenes 1/2a</i>	Ham	48 h at 5±3°C	2.8	+	
Dairy products	GL99	Cream cheese with garlic and herbs	LIS.4.24	<i>Listeria monocytogenes 1/2a</i>	Cheese meal	48 h at 5±3°C	1.6	+
	GL100	Cream cheese (pasteurized milk)	LIS.4.24	<i>Listeria monocytogenes 1/2a</i>	Cheese meal	48 h at 5±3°C	1.6	+
	GL101	Nature yoghurt (pasteurized milk)	LIS.4.24	<i>Listeria monocytogenes 1/2a</i>	Cheese meal	48 h at 5±3°C	1.6	+
	GL102	Montagnolo (cow milk pasteurized cheese)	LIS.4.58	<i>Listeria monocytogenes</i>	Not matured cow raw milk cheese	48 h at 5±3°C	1.4	+
	GL103	Organic Emmental (cow raw milk cheese)	LIS.4.58	<i>Listeria monocytogenes</i>	Not matured cow raw milk cheese	48 h at 5±3°C	1.4	+
	GL104	Tomme from Savoie (cow raw milk cheese)	LIS.4.58	<i>Listeria monocytogenes</i>	Not matured cow raw milk cheese	48 h at 5±3°C	1.4	+
	GL105	Comté (cow raw milk cheese)	LIS.4.60	<i>Listeria monocytogenes</i>	Cow raw milk cheese	48 h at 5±3°C	1.8	+
	GL106	Cœur de chèvre (goat raw milk cheese)	LIS.4.60	<i>Listeria monocytogenes</i>	Cow raw milk cheese	48 h at 5±3°C	1.8	+
	GL107	La croseta (goat raw milk cheese)	LIS.4.60	<i>Listeria monocytogenes</i>	Cow raw milk cheese	48 h at 5±3°C	1.8	+
	GL110	Ribot fermented milk	LIS.4.69	<i>Listeria monocytogenes</i>	Raw milk cheese	48 h at 5±3°C	1.0	+
	GL123	Cabri de Touraine cendré (goat raw milk cheese)	LIS.4.23	<i>Listeria monocytogenes 1/2a</i>	Cottage cheese	48 h at 5±3°C	3.3	+
	GL124	Butter (raw milk)	LIS.4.23	<i>Listeria monocytogenes 1/2a</i>	Cottage cheese	48 h at 5±3°C	3.3	+
	GL125	Sweet churned butter (raw milk)	LIS.4.23	<i>Listeria monocytogenes 1/2a</i>	Cottage cheese	48 h at 5±3°C	3.3	+
	GL126	Sweet salted churned butter (raw milk)	LIS.4.4	<i>Listeria monocytogenes 1/2a</i>	Zucchini goat cheese skewer	48 h at 5±3°C	2.3	+
	GL127	Mascarpone	LIS.4.4	<i>Listeria monocytogenes 1/2a</i>	Zucchini goat cheese skewer	48 h at 5±3°C	2.3	+
	GL128	Fermented skimmed ribot milk	LIS.4.4	<i>Listeria monocytogenes 1/2a</i>	Zucchini goat cheese skewer	48 h at 5±3°C	2.3	+
	GL129	Pasteurized semi-skimmed milk	LIS.4.46	<i>Listeria monocytogenes 3a</i>	Goat cheese sandwich	48 h at 5±3°C	0.3	+
GL130	Micro-filtered semi-skimmed milk	LIS.4.46	<i>Listeria monocytogenes 3a</i>	Goat cheese sandwich	48 h at 5±3°C	0.3	+	
GL131	Micro-filtered organic raw milk	LIS.4.46	<i>Listeria monocytogenes 3a</i>	Goat cheese sandwich	48 h at 5±3°C	0.3	+	
GL132	Cow raw milk	LIS.4.7	<i>Listeria monocytogenes 1/2a</i>	Ham-emental sandwich	48 h at 5±3°C	3.0	+	
GL133	Fermented ribot milk	LIS.4.7	<i>Listeria monocytogenes 1/2a</i>	Ham-emental sandwich	48 h at 5±3°C	3.0	+	
GL134	Fermented ribot milk	LIS.4.7	<i>Listeria monocytogenes 1/2a</i>	Ham-emental sandwich	48 h at 5±3°C	3.0	+	
GL165	Pistachio ice-cream	LIS.4.56	<i>Listeria monocytogenes</i>	Cow raw milk cheese	48 h at 5±3°C	2.8	+	
GL166	Coffee ice-cream	LIS.4.56	<i>Listeria monocytogenes</i>	Cow raw milk cheese	48 h at 5±3°C	2.8	+	

Category	Sample ID	Sample	Strain code	Strain	Origin	Seeding protocol	Inoculation level (CFU/test portion)	Global result
Seafood products	GL 375	Marinated tuna carpaccio	LIS.4.8	<i>Listeria monocytogenes</i> 1/2a	sandwich tuna egg surimi	48 h at 5±3°C	1.2	+
	GL 376	Anchovy and capers with vinegar	LIS.4.8	<i>Listeria monocytogenes</i> 1/2a	sandwich tuna egg surimi	48 h at 5±3°C	1.2	-
	GL 377	Rollmops	LIS.4.8	<i>Listeria monocytogenes</i> 1/2a	sandwich tuna egg surimi	48 h at 5±3°C	1.2	-
	GL 378	Monkfish fillet	LIS.4.12	<i>Listeria monocytogenes</i> 1/2a	Smoked salmon	48 h at 5±3°C	1.6	+
	GL 379	Red mullet fillet	LIS.4.12	<i>Listeria monocytogenes</i> 1/2a	Smoked salmon	48 h at 5±3°C	1.6	-
	GL 380	Whiting fillet	LIS.4.12	<i>Listeria monocytogenes</i> 1/2a	Smoked salmon	48 h at 5±3°C	1.6	+
	GL 381	Swordfish	LIS.4.15	<i>Listeria monocytogenes</i> 1/2a	Salmon tartar	48 h at 5±3°C	0.8	+
	GL 382	Cod fillet	LIS.4.15	<i>Listeria monocytogenes</i> 1/2a	Salmon tartar	48 h at 5±3°C	0.8	+
	GL 383	Plaice fillet	LIS.4.15	<i>Listeria monocytogenes</i> 1/2a	Salmon tartar	48 h at 5±3°C	0.8	+
	GL 387	Smoked trout	LIS.4.25	<i>Listeria monocytogenes</i> 1/2a	Fish and vegetables à la provençale	48 h at 5±3°C	0.4	+
	GL 388	Smoked trout offcuts	LIS.4.25	<i>Listeria monocytogenes</i> 1/2a	Fish and vegetables à la provençale	48 h at 5±3°C	0.4	+
	GL 389	Smoked salmon offcuts	LIS.4.25	<i>Listeria monocytogenes</i> 1/2a	Fish and vegetables à la provençale	48 h at 5±3°C	0.4	+
	GL 393	Tuna à la catalane	LIS.4.42	<i>Listeria monocytogenes</i> 3a	Smoked salmon	48 h at 5±3°C	0.2	+
GL 394	Salmon rillettes	LIS.4.42	<i>Listeria monocytogenes</i> 3a	Smoked salmon	48 h at 5±3°C	0.2	+	
GL 395	Parisian tuna salad	LIS.4.42	<i>Listeria monocytogenes</i> 3a	Smoked salmon	48 h at 5±3°C	0.2	+	
Vegetal products	GL 189	Entire frozen morels	LIS.4.4	<i>Listeria monocytogenes</i> 1/2a	Zucchini goat cheese skewer	48 h at 5±3°C	2.8	-
	GL 190	Pre-cooked lentils	LIS.4.4	<i>Listeria monocytogenes</i> 1/2a	Zucchini goat cheese skewer	48 h at 5±3°C	2.8	+
	GL 191	Zucchini purée	LIS.4.4	<i>Listeria monocytogenes</i> 1/2a	Zucchini goat cheese skewer	48 h at 5±3°C	2.8	+
	GL 192	Celery purée	LIS.4.5	<i>Listeria monocytogenes</i> 1/2a	Ham vegetables	48 h at 5±3°C	2.0	+
	GL 193	Split peas purée	LIS.4.5	<i>Listeria monocytogenes</i> 1/2a	Ham vegetables	48 h at 5±3°C	2.0	+
	GL 194	Pre-cooked potatoes	LIS.4.5	<i>Listeria monocytogenes</i> 1/2a	Ham vegetables	48 h at 5±3°C	2.0	+
	GL 195	Mung beans sprouts	LIS.4.10	<i>Listeria monocytogenes</i> 1/2a	Salad	48 h at 5±3°C	2.4	-
	GL 196	Vegetables mix for soup	LIS.4.10	<i>Listeria monocytogenes</i> 1/2a	Salad	48 h at 5±3°C	2.4	+
	GL 197	Packed red and white cabbage	LIS.4.10	<i>Listeria monocytogenes</i> 1/2a	Salad	48 h at 5±3°C	2.4	+
	GL 198	Entire frozen chanterelle mushrooms	LIS.4.20	<i>Listeria monocytogenes</i> 1/2a	Sandwich bacon vegetables	48 h at 5±3°C	1.2	-
	GL 199	Frozen entire porcini mushrooms	LIS.4.20	<i>Listeria monocytogenes</i> 1/2a	Sandwich bacon vegetables	48 h at 5±3°C	1.2	+
	GL 200	Strawberries	LIS.4.20	<i>Listeria monocytogenes</i> 1/2a	Sandwich bacon vegetables	48 h at 5±3°C	1.2	-
	GL 201	Pre-cooked cauliflower	LIS.4.17	<i>Listeria monocytogenes</i> 1/2a	Vegetables	48 h at 5±3°C	1.4	+
	GL 202	Packed lamb's lettuce	LIS.4.17	<i>Listeria monocytogenes</i> 1/2a	Vegetables	48 h at 5±3°C	1.4	-
	GL 203	Flat parsley	LIS.4.17	<i>Listeria monocytogenes</i> 1/2a	Vegetables	48 h at 5±3°C	1.4	+
	GL 204	Basil	LIS.4.18	<i>Listeria monocytogenes</i> 1/2a	Vegetables salad	48 h at 5±3°C	0.8	+
	GL 205	Tarragon	LIS.4.18	<i>Listeria monocytogenes</i> 1/2a	Guiney fowl	48 h at 5±3°C	0.8	-
	GL 206	Chives	LIS.4.18	<i>Listeria monocytogenes</i> 1/2a	Vegetables salad	48 h at 5±3°C	0.8	-
	GL 358	Cherry tomatoes	LIS.4.76	<i>Listeria monocytogenes</i>	Salad	48 h at 5±3°C	0.6	+
	GL 590	Pineapple	LIS.4.79	<i>Listeria monocytogenes</i>	Deep-frozen peeled beans	48 h at 5±3°C	2.0	+
GL 591	Apples	LIS.4.79	<i>Listeria monocytogenes</i>	Deep-frozen peeled beans	48 h at 5±3°C	2.0	+	
GL 592	Melon	LIS.4.79	<i>Listeria monocytogenes</i>	Deep-frozen peeled beans	48 h at 5±3°C	2.0	+	

Category	Sample ID	Sample	Strain code	Strain	Origin	Seeding protocol	Inoculation level (CFU/test portion)	Global result
Composite foods	GL 254	Pear pie	LIS.4.20	<i>Listeria monocytogenes 1/2a</i>	Sandwich bacon vegetables	48 h at 5±3°C	0.6	-
	GL 255	Apricot pie	LIS.4.20	<i>Listeria monocytogenes 1/2a</i>	Sandwich bacon vegetables	48 h at 5±3°C	0.6	+
	GL 256	Cherry cobbler	LIS.4.20	<i>Listeria monocytogenes 1/2a</i>	Sandwich bacon vegetables	48 h at 5±3°C	0.6	-
	GL 257	Apple pie	LIS.4.20	<i>Listeria monocytogenes 1/2a</i>	Sandwich bacon vegetables	48 h at 5±3°C	0.6	+
	GL 258	Flan	LIS.4.20	<i>Listeria monocytogenes 1/2a</i>	Sandwich bacon vegetables	48 h at 5±3°C	0.6	+
	GL 259	Mirabelle pie	LIS.4.20	<i>Listeria monocytogenes 1/2a</i>	Sandwich bacon vegetables	48 h at 5±3°C	0.6	+
	GL 260	Salad ham, vegetables, emmental	LIS.4.39	<i>Listeria monocytogenes 1/2c</i>	Salmon tartar	48 h at 5±3°C	2.4	+
	GL 261	Salad tuna, pasta, vegetables	LIS.4.39	<i>Listeria monocytogenes 1/2c</i>	Salmon tartar	48 h at 5±3°C	2.4	+
	GL 262	Salad chicken vegetables	LIS.4.39	<i>Listeria monocytogenes 1/2c</i>	Salmon tartar	48 h at 5±3°C	2.4	+
	GL 263	Chicken tabouleh	LIS.4.42	<i>Listeria monocytogenes 3a</i>	Smoked salmon	48 h at 5±3°C	7.0	+
	GL 264	Torti surimi	LIS.4.42	<i>Listeria monocytogenes 3a</i>	Smoked salmon	48 h at 5±3°C	7.0	+
	GL 265	Piémontaise salad	LIS.4.42	<i>Listeria monocytogenes 3a</i>	Smoked salmon	48 h at 5±3°C	7.0	+
	GL 266	Pizza 4 cheese	LIS.4.46	<i>Listeria monocytogenes 3a</i>	Goat cheese sandwich	48 h at 5±3°C	2.2	+
	GL 267	Pizza ham emmental	LIS.4.46	<i>Listeria monocytogenes 3a</i>	Goat cheese sandwich	48 h at 5±3°C	2.2	-
	GL 268	Fusilli carbonara	LIS.4.46	<i>Listeria monocytogenes 3a</i>	Goat cheese sandwich	48 h at 5±3°C	2.2	+
	GL 269	Fusilli with cheese	LIS.4.77	<i>Listeria monocytogenes</i>	Tuna vegetables sandwich	48 h at 5±3°C	7.0	+
	GL 270	Noodles chicken vegetables	LIS.4.77	<i>Listeria monocytogenes</i>	Tuna vegetables sandwich	48 h at 5±3°C	7.0	+
	GL 271	Bolognese penne	LIS.4.77	<i>Listeria monocytogenes</i>	Tuna vegetables sandwich	48 h at 5±3°C	7.0	+
	GL 314	Coconut flan	LIS.4.6	<i>Listeria monocytogenes 1/2a</i>	Ham emmental sandwich	48 h at 5±3°C	0.6	+
	GL 315	Coffee flavored custard pastry	LIS.4.6	<i>Listeria monocytogenes 1/2a</i>	Ham emmental sandwich	48 h at 5±3°C	0.6	+
	GL 316	Cookie	LIS.4.6	<i>Listeria monocytogenes 1/2a</i>	Ham emmental sandwich	48 h at 5±3°C	0.6	-
	GL 317	Grape flan	LIS.4.7	<i>Listeria monocytogenes 1/2a</i>	Ham emmental sandwich	48 h at 5±3°C	0.6	+
	GL 318	Salad cabbage, ham, comté	LIS.4.7	<i>Listeria monocytogenes 1/2a</i>	Ham emmental sandwich	48 h at 5±3°C	0.6	-
	GL 319	Salad pineapple, carrot, surimi	LIS.4.7	<i>Listeria monocytogenes 1/2a</i>	Ham emmental sandwich	48 h at 5±3°C	0.6	+
GL 320	Prawn salad with mandarins	LIS.4.8	<i>Listeria monocytogenes 1/2a</i>	Tuna egg surimi sandwich	48 h at 5±3°C	1.8	+	
GL 321	Salad potatoes sausages	LIS.4.8	<i>Listeria monocytogenes 1/2a</i>	Tuna egg surimi sandwich	48 h at 5±3°C	1.8	-	
GL 322	Chicken tabouleh	LIS.4.8	<i>Listeria monocytogenes 1/2a</i>	Tuna egg surimi sandwich	48 h at 5±3°C	1.8	+	

Category	Sample ID	Sample	Strain code	Strain	Origin	Seeding protocol	Inoculation level (CFU/test portion)	Global result
Environmental samples	GL 457	Swab 13	LIS.4.2	<i>Listeria monocytogenes</i>	Environment	48 h at 5±3°C	2.7	+
	GL 458	Sponge 1	LIS.4.2	<i>Listeria monocytogenes</i>	Environment	48 h at 5±3°C	2.7	+
	GL 459	Sponge 2	LIS.4.2	<i>Listeria monocytogenes</i>	Environment	48 h at 5±3°C	2.7	+
	GL 460	Sponge 3	LIS.4.16	<i>Listeria monocytogenes 1/2a</i>	Surface sample sewage	48 h at 5±3°C	1.7	+
	GL 461	Sponge 4	LIS.4.16	<i>Listeria monocytogenes 1/2a</i>	Surface sample sewage	48 h at 5±3°C	1.7	+
	GL 462	Sponge 5	LIS.4.16	<i>Listeria monocytogenes 1/2a</i>	Surface sample sewage	48 h at 5±3°C	1.7	+
	GL 463	Sponge 6	LIS.4.16	<i>Listeria monocytogenes 1/2a</i>	Surface sample sewage	48 h at 5±3°C	1.7	+
	GL 466	Process water 3	LIS.4.50	<i>Listeria monocytogenes 4b</i>	Surface sample salmon	48 h at 5±3°C	2.1	+
	GL 467	Process water 4	LIS.4.50	<i>Listeria monocytogenes 4b</i>	Surface sample salmon	48 h at 5±3°C	2.1	+
	GL 470	Process water 7	LIS.4.44	<i>Listeria monocytogenes 3a</i>	Surface sample	48 h at 5±3°C	2.0	+
	GL 471	Process water 8	LIS.4.44	<i>Listeria monocytogenes 3a</i>	Surface sample	48 h at 5±3°C	2.0	+
	GL 472	Process water 9	LIS.4.50	<i>Listeria monocytogenes 4b</i>	Surface sample salmon	48 h at 5±3°C	3.0	+
	GL 473	Process water 10	LIS.4.57	<i>Listeria monocytogenes</i>	Goat milk filter	48 h at 5±3°C	3.0	+
	GL 474	Process water 11	LIS.4.57	<i>Listeria monocytogenes</i>	Goat milk filter	48 h at 5±3°C	3.0	-
	GL 475	Swab 14	LIS.4.2	<i>Listeria monocytogenes</i>	Environment	48 h at 5±3°C	2.7	+
	GL 503	Dust 11	LIS.4.67	<i>Listeria monocytogenes 1/2 b</i>	Raw milk	48 h at 5±3°C	2.1	-
	GL 504	Dust 12	LIS.4.67	<i>Listeria monocytogenes 1/2 b</i>	Raw milk	48 h at 5±3°C	2.1	-
	GL 505	Dust 13	LIS.4.67	<i>Listeria monocytogenes 1/2 b</i>	Raw milk	48 h at 5±3°C	2.1	-
	GL 506	Dust 14	LIS.4.67	<i>Listeria monocytogenes 1/2 b</i>	Raw milk	48 h at 5±3°C	2.1	-
	GL 507	Dust 15	LIS.4.67	<i>Listeria monocytogenes 1/2 b</i>	Raw milk	48 h at 5±3°C	2.1	-
	GL 566	Process water 35	LIS.4.68	<i>Listeria monocytogenes</i>	Surface sample	48 h at 5±3°C	2.8	+
	GL 567	Process water 36	LIS.4.68	<i>Listeria monocytogenes</i>	Surface sample	48 h at 5±3°C	2.8	+
	GL 568	Process water 37	LIS.4.68	<i>Listeria monocytogenes</i>	Surface sample	48 h at 5±3°C	2.8	+
	GL 569	Process water 38	LIS.4.68	<i>Listeria monocytogenes</i>	Surface sample	48 h at 5±3°C	2.8	+
	GL 570	Process water 39	LIS.4.68	<i>Listeria monocytogenes</i>	Surface sample	48 h at 5±3°C	2.8	+
	GL 571	Process water 40	LIS.4.2	<i>Listeria monocytogenes</i>	Environment	48 h at 5±3°C	2.0	+
	GL 572	Process water 41	LIS.4.2	<i>Listeria monocytogenes</i>	Environment	48 h at 5±3°C	2.0	+
	GL 575	Dust 24	LIS.4.57	<i>Listeria monocytogenes</i>	Goat milk filter	48 h at 5±3°C	6.4	+
	GL 576	Sponge 29	LIS.4.50	<i>Listeria monocytogenes 4b</i>	Surface sample salmon	48 h at 5±3°C	3.2	+
	GL 577	Sponge 30	LIS.4.50	<i>Listeria monocytogenes 4b</i>	Surface sample salmon	48 h at 5±3°C	3.2	+
	GL 578	Sponge 31	LIS.4.50	<i>Listeria monocytogenes 4b</i>	Surface sample salmon	48 h at 5±3°C	3.2	+
	GL 579	Sponge 32	LIS.4.16	<i>Listeria monocytogenes 1/2a</i>	Surface sample sewage	48 h at 5±3°C	2.2	+
	GL 580	Sponge 33	LIS.4.16	<i>Listeria monocytogenes 1/2a</i>	Surface sample sewage	48 h at 5±3°C	2.2	+
	GL 581	Sponge 34	LIS.4.57	<i>Listeria monocytogenes</i>	Goat milk filter	48 h at 5±3°C	6.4	+
	GL 588	Dust 25	LIS.4.57	<i>Listeria monocytogenes</i>	Goat milk filter	48 h at 5±3°C	6.4	+
	GL 589	Dust 26	LIS.4.57	<i>Listeria monocytogenes</i>	Goat milk filter	48 h at 5±3°C	6.4	+
	GL 593	Swab 42	LIS.4.44	<i>Listeria monocytogenes 3a</i>	Surface sample	48 h at 5±3°C	1.8	+
	GL 594	Swab 43	LIS.4.44	<i>Listeria monocytogenes 3a</i>	Surface sample	48 h at 5±3°C	1.8	+
	GL 595	Swab 44	LIS.4.44	<i>Listeria monocytogenes 3a</i>	Surface sample	48 h at 5±3°C	1.8	+
	GL 596	Swab 45	LIS.4.44	<i>Listeria monocytogenes 3a</i>	Surface sample	48 h at 5±3°C	1.8	+

APPENDIX 4A

SENSITIVITY

RAW RESULTS

Caption:

ST : sample type
SN : sample number
: sample identity
◊ : level determined by 3 to 5 enumerations
sp : spiking
se : seeding
nc : naturally contaminated
cm: contamination by mixture
+ / Pos : positive result
- / Neg : negative result
/ : test not realized
∅ : absence of colonies
PA : positive agreement
NA : negative agreement
PD : positive deviation
ND : negative deviation
FN : false negative result
FP : false positive result
PP: presumed positive result before confirmation
A : absence
P : presence
0 / 1 / 2 / 3 / 4 : level of typical flora, from absence to high
∅ / L / M / H : level of annex flora, from absence to high
I : result after re-isolation
(XXX) : number of typical colonies
L.m : Listeria monocytogenes
L.w : Listeria welshimeri
L.in: Listeria innocua
L.iv: Listeria ivanovii
Confirmation : streaking on selective medium + ISO 11290-1 confirmation
Conf. 1 : streaking on selective medium + visual reading
Conf. 2 : streaking on selective medium + API Listeria
Conf. 3 : streaking on selective medium + RAPIDEC L-mono
Conf. 4 : streaking on selective medium + Fast Rhamnose
Conf. 5 : streaking on selective medium + ISO 11290-1 confirmation (case n°1)
chromID L. mono: w=white colonies / b=blue colonies

CP: Number of amplification cycles necessary to obtain a statistically significant fluorescent signal with regard to the background noise
MP: Temperature for which 50 % of the double-strand DNA is separated

MEAT PRODUCTS

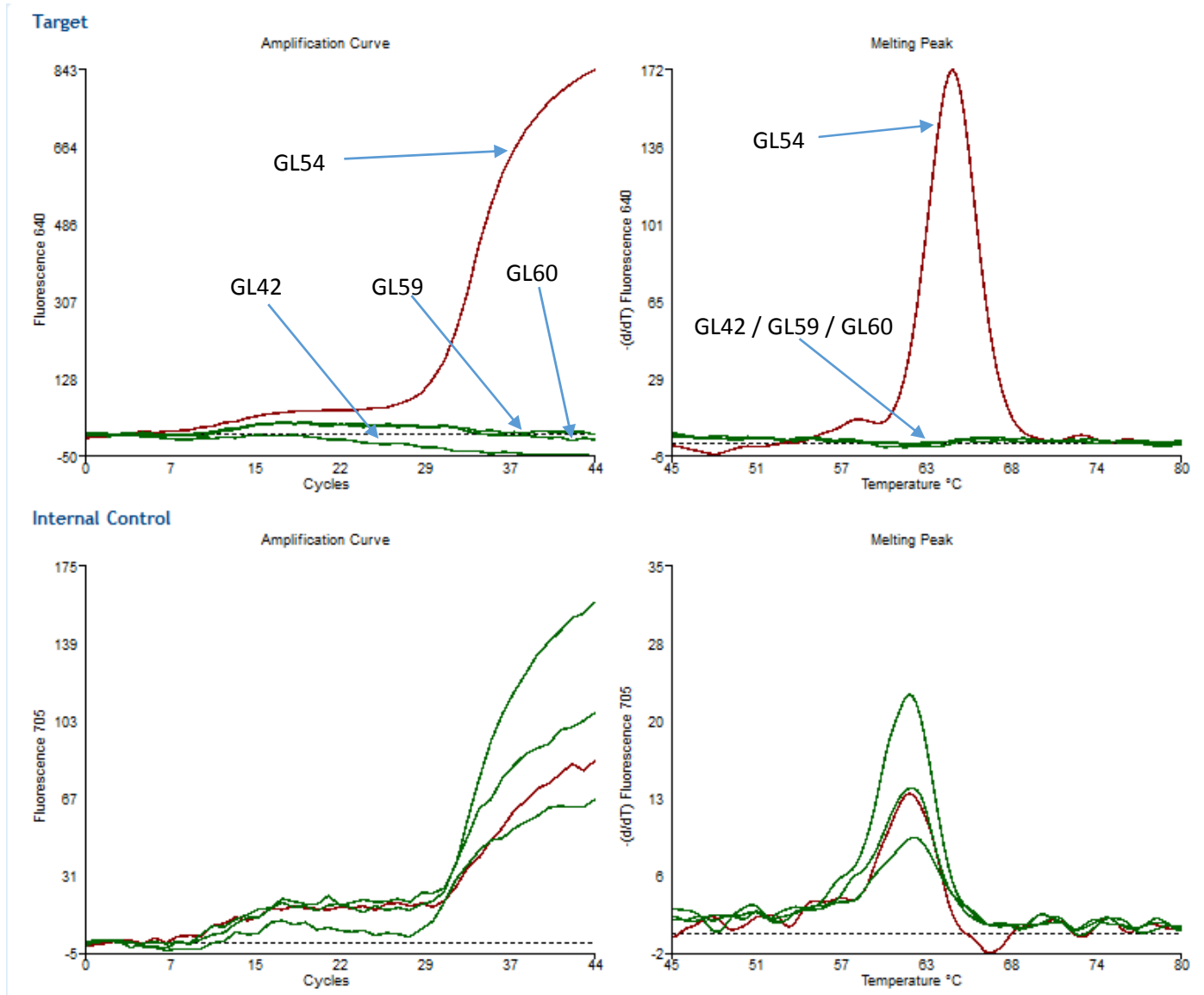
ST	SN	#	Sample	Contamination strain or serovar, type (nc.sp.se or cm) and level (CFU/25 g)	RM: NF EN ISO 11290-1 (*)				AM: GENE UP								AM: GENE UP after storage of the lysates 3 days at 5°C			AM: GENE UP after storage 3 days at 5°C				Confirmation ISO 16140-2 on MA		Concordance RM /AM							
					Half Fraser		Fraser		Confirmation	Final result	CP	MP	GENE UP result	Conf. 1		Conf. 2	Conf. 3	Conf. 4	Conf. 5	Final result	CP	MP	GENE UP	CP	MP	GENE UP result	Conf. 1		Conf. 5	Final result	Final result	After a 3-day storage at 5°C	vs suppl. conf.
					ALOA	PALCAM	ALOA	PALCAM						ALOA	Conf. 2												ALOA	ALOA					
b-46	GL52		Raw pork tenderloin	/ / / /	0 L 0 M 0 L 0 L -	A	0,00 0,00	-	3h- L	- (L.w)	/ / /	- (L.w)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-47	GL53		Raw beef bavette	/ / / /	0 L 0 M 0 L 0 L -	A	0,00 0,00	-	0 L	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a+48	GL54		Raw horse rumsteak	/ / nc / /	1h+ Ø 0 L 4h+ 1h- Ø 4 Ø + (L.w + L.m)	P	30,18 64,31	+	4h+ Ø	+ (L.m)	L.m	L.m	+ (L.m)	P	28,32 65,01	+	26,28 64,56	+	4h+ Ø	P	/ / /	PA	PA	/									
b-49	GL55		Raw turkey filet mignon	/ / / /	3h- L 3 L 2h- Ø 3 Ø - (L.w)	A	0,00 0,00	-	3h- M	- (L.in)	/ / /	- (L.in)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
c-50	GL56		Smoked sausages	/ / / /	2h- L 0 H 3h- Ø 3 M - (L.w)	A	0,00 0,00	-	3h- L	- (L.in)	/ / /	- (L.in)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
c-51	GL57		Smoked lardons	/ / / /	0 L 0 L 0 Ø 0 Ø -	A	0,00 0,00	-	0 M	- (L.w)	/ / /	- (L.w)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
b-52	GL58		Pork meatloaf	/ / / /	0 Ø 0 L 0 L 0 L -	A	0,00 0,00	-	0 Ø	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
c-53	GL59		Smoked salami	/ / / /	0 Ø 0 L 0 Ø 0 Ø -	A	9,68 0,00	-	0 L	- (L.in)	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
c-54	GL60		Smoked bacon	/ / / /	0 L 0 L 4h- Ø 4 Ø - (L.w)	A	9,50 0,00	-	3h- M	- (L.w)	/ / /	- (L.w)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
b-55	GL61		Small pork sausages	/ / / /	0 Ø 0 Ø 0 Ø 0 Ø -	A	0,00 0,00	-	0 M	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
b-56	GL62		Pork rillettes	/ / / /	0 Ø 0 Ø 0 Ø 0 Ø -	A	0,00 0,00	-	0 Ø	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-57	GL135		Raw marinated beef (nut oil, balsamic vinegar)	/ / / /	0 L 0 L 0 Ø 0 Ø -	A	0,00 0,00	-	0 L	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-58	GL136		Raw marinated beef (lemon olive oil)	/ / / /	0 Ø 0 Ø 0 Ø 0 Ø -	A	0,00 0,00	-	0 Ø	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-59	GL137		Raw marinated beef (parmesiano, tomatoes)	/ / / /	0 Ø 0 Ø 0 Ø 0 Ø -	A	0,00 0,00	-	0 Ø	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a+60	GL138		Raw turkey filet mignon	/ / nc / /	2h+/1h- L 3 L 3h+ 2h- Ø 2 Ø + (L.in + L.m)	P	24,16 64,72	+	4h+ 1h- L	+ (L.in + L.m)	L.m	L.m	+ (L.in + L.m)	P	23,56 64,75	+	23,76 65,04	+	4h+ 2h- Ø	P	/ / /	PA	PA	/									
a-61	GL139		Raw turkey scallop	/ / / /	2h- L 2 L 3h- Ø 3 Ø - (L.w)	A	0,00 0,00	-	4h- L	- (L.w)	/ / /	- (L.w)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-62	GL140		Raw chicken fillet	/ / / /	0 L 0 M 0 Ø 0 Ø -	A	0,00 0,00	-	0 H	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a+63	GL141		Raw chicken scallop	/ / nc / /	2h- L 1 L 2h- L 2 L - (L.w)	A	25,00 65,18	+	4h+ Ø	+ (L.w + L.m)	L.m	L.m	+ (L.w + L.m)	P	23,64 64,76	+	22,32 65,01	+	4h+ L	P	/ / /	PD	PD	/									
a-64	GL142		Raw beef tenderloin	/ / / /	0 Ø 0 H 0 Ø 0 M -	A	0,00 0,00	-	3h- Ø	- (L.w)	/ / /	- (L.w)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-65	GL143		Raw beef sirloin	/ / / /	1h- Ø 1 L 3h- Ø 3 Ø - (L.w)	A	0,00 0,00	-	0 M	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a+66	GL144		Raw lamb	/ / nc / /	2h+ Ø 2 L 3h+ 2h- L 4 L + (L.in + L.m)	P	27,83 64,94	+	3h+ 1h- Ø	+ (L.in + L.m)	L.m	L.m	+ (L.in + L.m)	P	22,93 64,85	+	27,18 65,17	+	2h+ 1h- Ø	P	/ / /	PA	PA	/									
a-67	GL145		Raw beef tenderloin	/ / / /	0 Ø 0 L 0 Ø 0 L -	A	0,00 0,00	-	0 L	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-68	GL146		Raw beef meat	/ / / /	0 Ø 0 L 0 Ø 0 L -	A	0,00 0,00	-	0 M	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-69	GL147		Raw beef rumsteak	/ / / /	0 L 0 L 0 Ø 0 L -	A	0,00 0,00	-	0 M	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-70	GL148		Raw horse striploin	/ / / /	0 Ø 0 M 0 Ø 0 L -	A	0,00 0,00	-	0 M	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a+71	GL149		Sliced raw horse	/ / nc / /	2h+ Ø 3 L 3h+ 1h- Ø 4 Ø + (L.in + L.m)	P	0,00 0,00	-	3h- Ø	- (L.in)	/ / /	- (L.in)	A	0,00 0,00	+	0,00 0,00	-	2h- Ø	A	-	A	ND	ND	ND									
a+72	GL150		Raw lamb shoulder	/ / nc / /	1h+ L 1 L 3h+ 1h- M 3 M + (L.in + L.m)	P	29,56 63,24	+	3h+ 2h- M	+ (L.in + L.m)	L.m	L.m	+ (L.in + L.m)	P	29,54 64,98	+	29,51 65,39	+	2h+ 1h- L	P	/ / /	PA	PA	/									
a-73	GL151		Raw veal steaks	/ / / /	1h- Ø 1 L 3h- L 3 L - (L.w)	A	0,00 0,00	-	4h- Ø	- (L.w)	/ / /	- (L.w)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-74	GL152		Raw beef rib steak	/ / / /	0 Ø 0 L 0 Ø 0 M -	A	0,00 0,00	-	0 L	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-75	GL153		Raw pork meat	/ / / /	1h- Ø 1 M 4h- Ø 4 Ø - (L.w)	A	0,00 0,00	-	0 M	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-76	GL154		Raw pork loin chop	/ / / /	0 L 0 L 0 L 0 M -	A	0,00 0,00	-	0 L	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-77	GL155		Raw lamb chops	/ / / /	0 Ø 0 L 0 Ø 0 L -	A	0,00 0,00	-	0 L	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-78	GL156		Raw veal chop	/ / / /	0 Ø 0 L 0 Ø 0 L -	A	0,00 0,00	-	0 M	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-79	GL157		Raw pork chop	/ / / /	1h- L 1 L 4h- Ø 4 Ø - (L.w)	A	0,00 0,00	-	2h- Ø	- (L.w)	/ / /	- (L.w)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a-80	GL158		Raw pork ribs	/ / / /	1h- L 1 M 3h- L 3 L - (L.w)	A	0,00 0,00	-	4h- Ø	- (L.w)	/ / /	- (L.w)	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
a+81	GL159		Raw beef tenderloin	LIS.4.11 se 1,6	0 Ø 0 L 0 Ø 0 L -	A	23,96 64,94	+	4h+ Ø	+ (L.m)	L.m	L.m	+ (L.m)	P	24,01 64,81	+	19,49 65,01	+	4h+ Ø	P	/ / /	PD	PD	/									
a+82	GL160		Raw turkey filet mignon	LIS.4.11 se 1,6	2h+/1h- Ø 3 Ø 1h+/2h- Ø 3 Ø + (L.m)	P	23,14 64,78	+	4h+ 1h- Ø	+ (L.in + L.m)	L.m	L.m	+ (L.in + L.m)	P	22,53 64,83	+	22,62 64,80	+	4h+ Ø	P	/ / /	PA	PA	/									
ch 83	GL161		Speck	LIS.4.11 se 1,6	0 Ø 0 L 1h+/2h- Ø 3 Ø + (L.m)	P	0,00 0,00	-	2h- L	- (L.in)	/ / /	- (L.in)	A	0,00 0,00	-	0,00 0,00	-	2h- M	A	-	A	ND	ND	ND									
ch 84	GL162		Smoked salami	LIS.4.26 se 2,8	0 Ø 0 Ø 0 Ø 0 Ø -	A	0,00 0,00	-	0 L	/ / /	/ / /	/ / /	A	0,00 0,00	-	/ / /	/ / /	-	A	NA	/	NA											
c-85	GL163		Ham without rind	LIS.4.26 se 2,8	0 Ø 0 Ø 0 Ø 0 Ø -	A	24,86 64,91	+	3h+ Ø	+ (L.m)	L.m	L.m	+ (L.m)	P	25,13 64,90	+	22,32 64,80	+	3h+ Ø	P	/ / /	PD	PD	/									
c+86	GL164		Ham with rind	LIS.4.26 se 2,8	1h+/1h- Ø 1 Ø 3h+ Ø 3 Ø + (L.m)	P	20,85 64,91	+	4h+ Ø	+ (L.m)	L.m	L.m	+ (L.m)	P	21,14 64,86	+	16,92 64,81	+	4h+ Ø	P	/ / /	PA	PA	/									

APPENDIX 4B

AMPLIFICATION CURVES FOR SAMPLES: GL59, GL60, GL88, GL94, GL95, GL96, GL106 and GL132

Samples GL59 and GL60

Curves for samples GL54 (positive) and GL42 (negative) are shown to better appreciate the curves of samples GL59 and GL60.



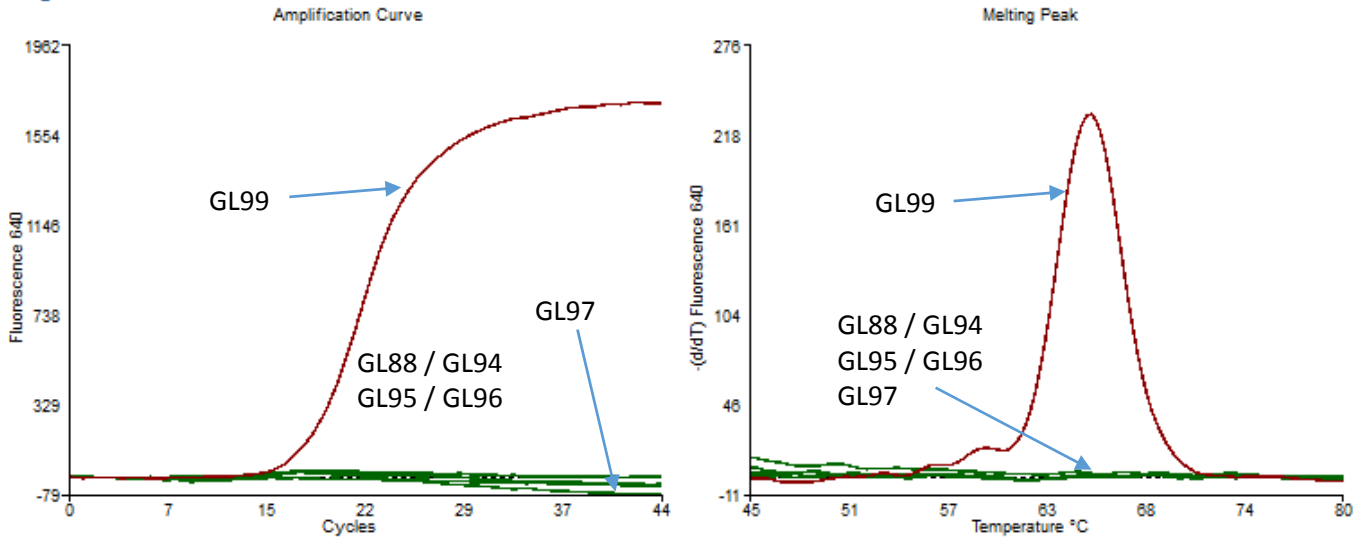
Assay	2	Sample ID	Res: 3	CP	TM (°)	Ir	Status	M	C	R	C	We 1	Tz
LMO		GEN 42	—	0	0	Succ	✓					D9	Nega
LMO		GEN 54	+	30.18	64.31	Succ	✓					H10	Positi
LMO		GEN 59	—	9.68	0	Succ	✓					E11	Nega
LMO		GEN 60	—	9.5	0	Succ	✓					F11	Nega

For samples GL59 and GL60, a CP is detected by the software whereas no amplification is visible on the curves. As no melting peak is detected for these samples, the software concludes to a negative result.

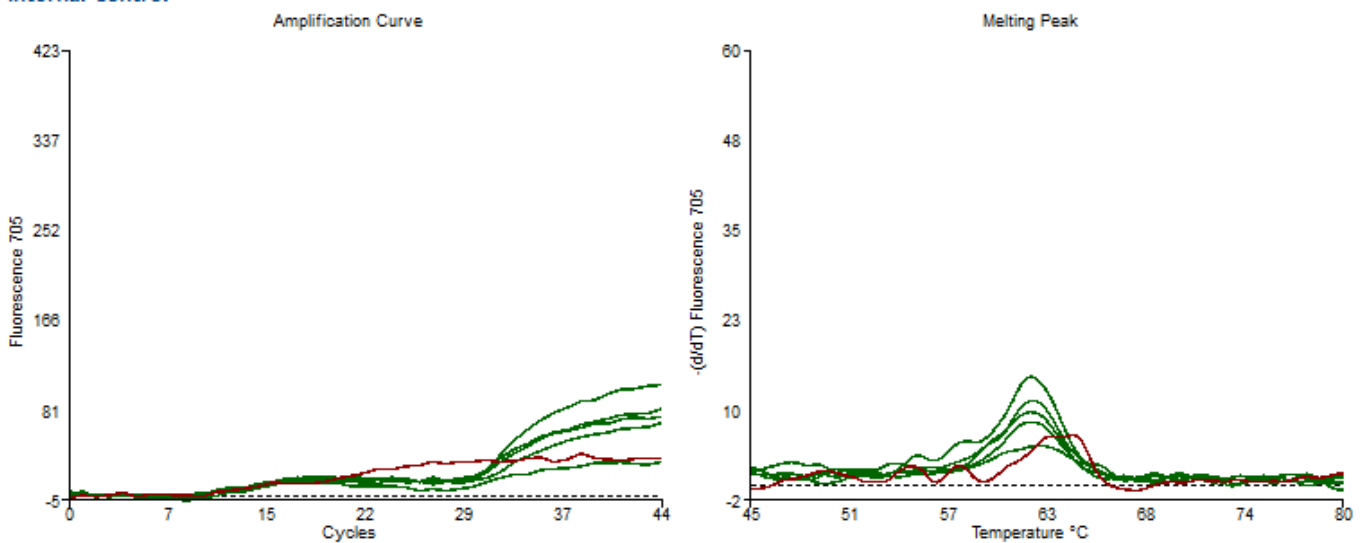
Samples GL88, GL94, GL95 and GL96

Curves for samples GL99 (positive) and GL97 (negative) are shown to better appreciate the curves of samples GL88, GL94, GL95 and GL96.

Target



Internal Control

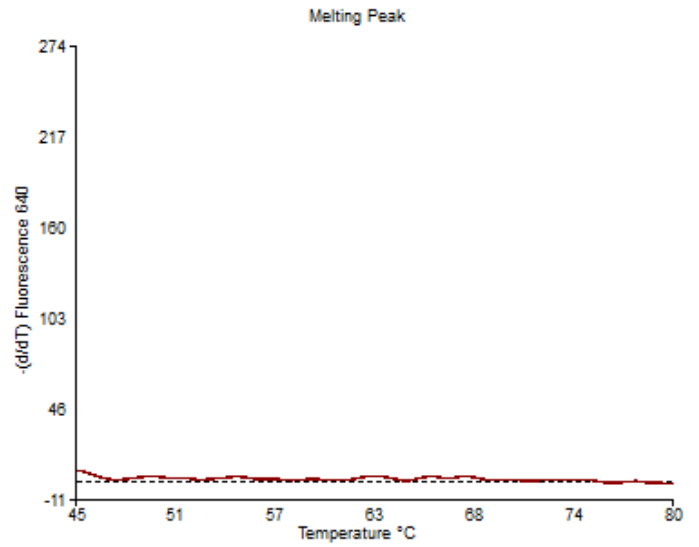
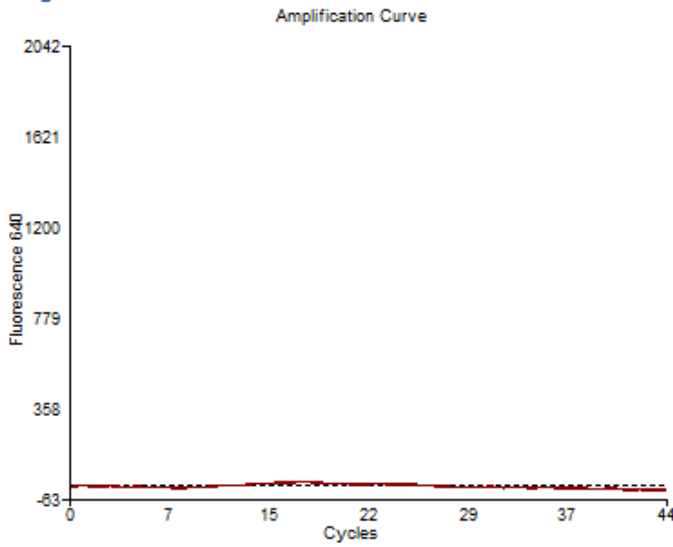


Assay	2	Sample ID	Res 3	CP	TM (°)	Ir	Status	M	C	R	C	We 1	Tz
LMO		GEN 88	—	10.53	0	Succ	✓✓					B9	Nega
LMO		GEN 94	—	10.25	0	Succ	✓✓					H9	Nega
LMO		GEN 95	—	11.43	0	Succ	✓✓					A10	Nega
LMO		GEN 96	—	10.38	0	Succ	✓✓					B10	Nega
LMO		GEN 97	—	0	0	Succ	✓✓					C10	Nega
LMO		GEN 99	+	17.92	65.11	Succ	✓✓					E10	Positi

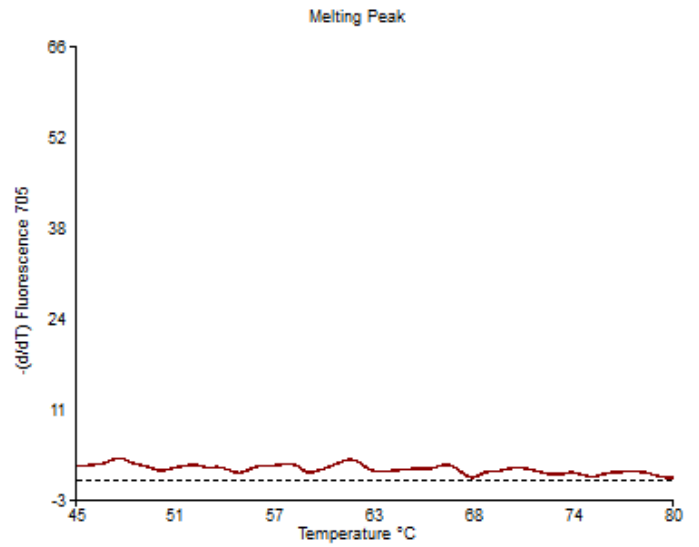
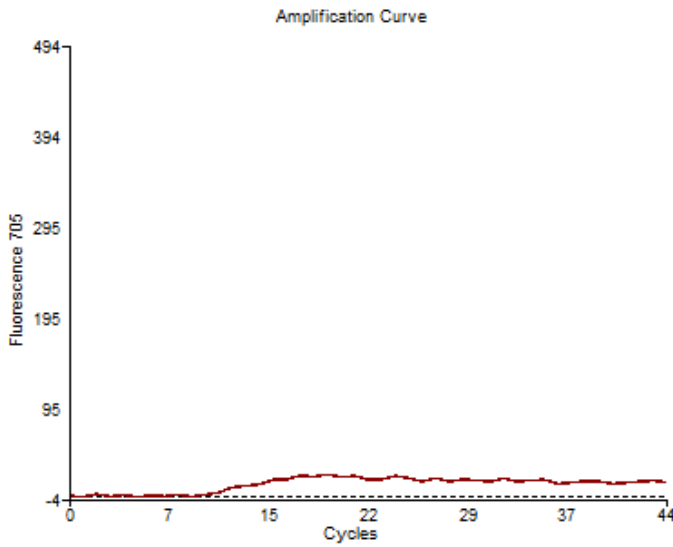
For samples GL88, GL94, GL95 and GL96, a CP is detected by the software whereas no amplification is visible on the curves. As no melting peak is detected for these samples, the software concludes to a negative result.

Sample GL106

Target



Internal Control



Assay	2	Sample ID	Res 3	CP	TM (°)	Ir	Status	M	C	R	C	We 1	Ti
LMO		GEN 106	+	0	53.1	Failu	✓					B12	Positi

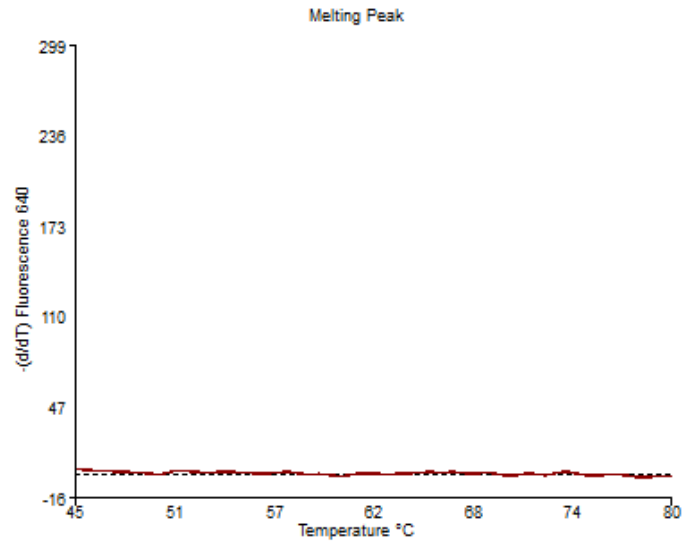
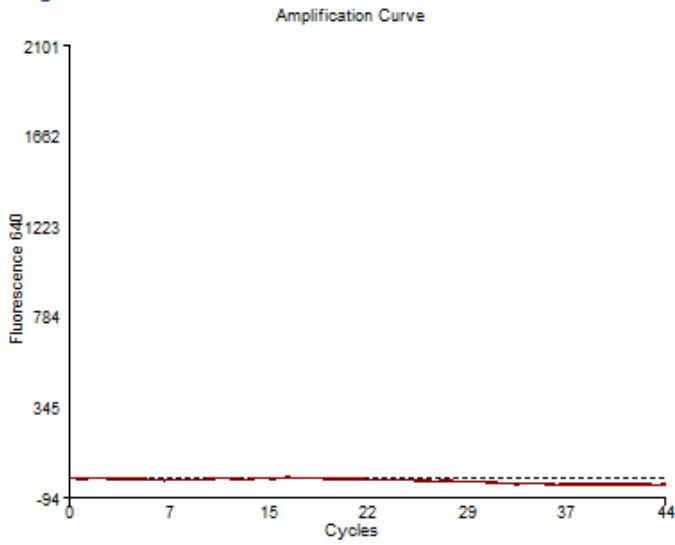
No CP is observed or detected. No MP is observed but a signal is detected.

This sample should have given an inhibited result as the internal control shows no melting peak. But, as a melting peak is detected for the target, a positive result is given.

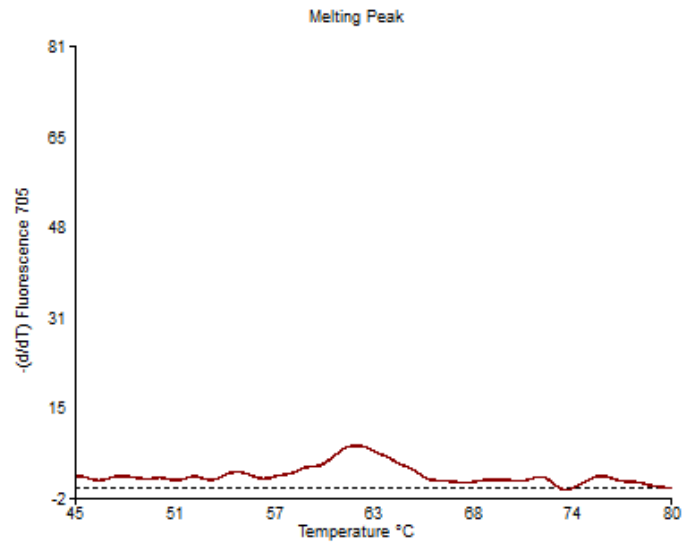
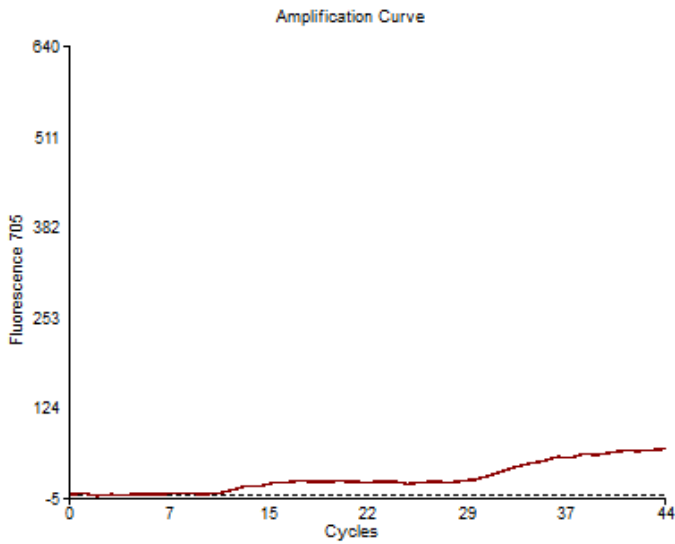
After retest from the stored lysate, a clear negative result is obtained (CP: 0 / MP: 0).

Sample GL132

Target



Internal Control



Assay	2	Sample ID	Res: 3	CP	TM (°)	Ir	Status	M	C	R	C	We 1	Ts
LMO		GEN 132	+	0	51.32	Success	✓					F12	Positi

No CP is observed or detected. No MP is observed but a signal is detected.

After retest from the stored lysate, a clear negative result is obtained (CP: 0 / MP: 0).

APPENDIX 4C

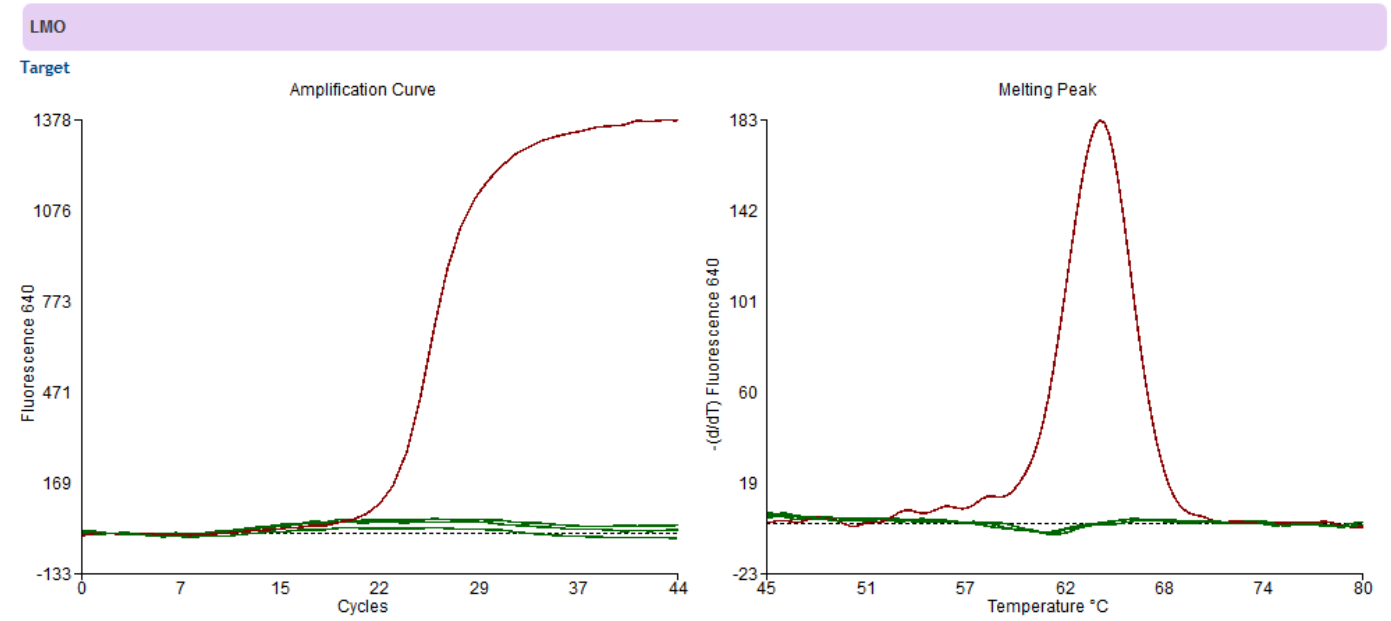
AMPLIFICATION CURVES FOR SAMPLES:

Negative result with a CP value and no MP:

Samples: GL 257, GI 282, GL 489, GL 494, GL 495, GL 503, GL 544, GL 545, GL 557, GL 558, GL 559, GL 562, GL 564, GL 565, GL 569, GL 574, GL 582, GL 583, GL 585, GL 586, GL 495 Lysate stored at 5°C, 519 broth stored at 5°C, GL 547 broth stored at 5°C, GL 552 broth stored at 5°C, GL 554 broth stored at 5°C, GL 555 broth stored at 5°C, GL 556 broth stored at 5°C, GL 557 broth stored at 5°C, GL 565 broth stored at 5°C

Example of curves for negative result:

GL 494, GL 495, GL 503 (example of positive result: GL 500)

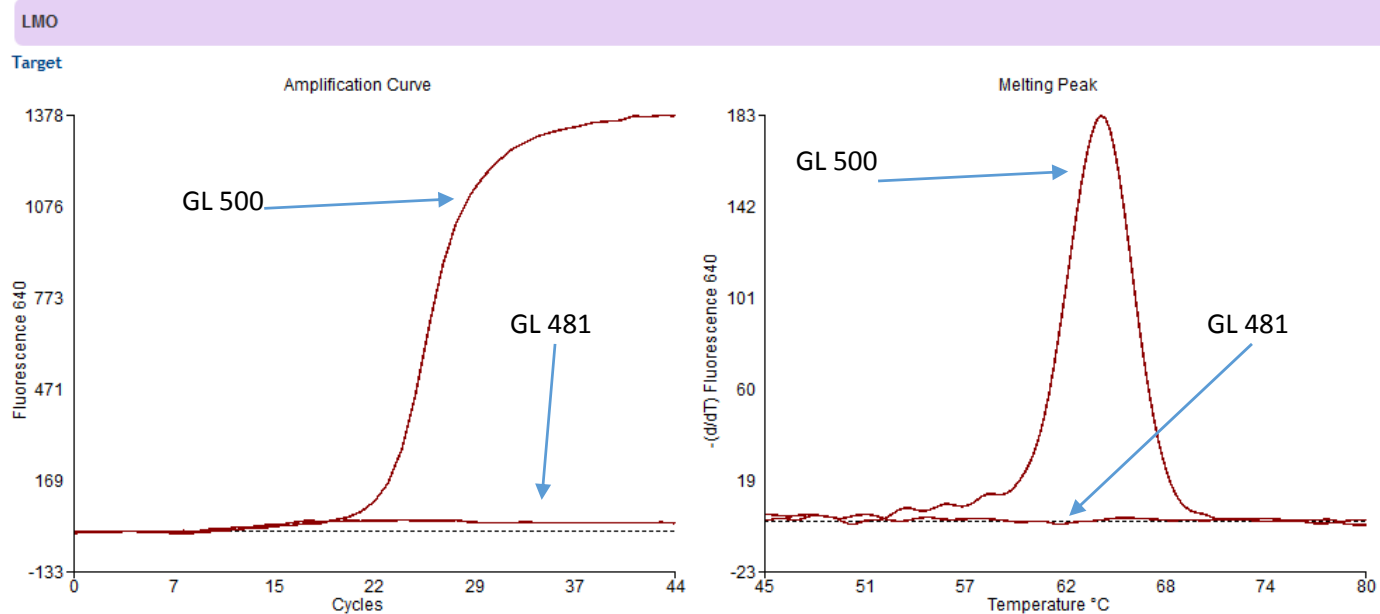


Assay	2	Sample ID	Resi 3	CP 4	TM (°)	In	Status
LMO		GEN 494	—	8.85	0	Succ	<input type="checkbox"/>
LMO		GEN 495	—	8.98	0	Succ	<input type="checkbox"/>
LMO		GEN 500	+	22.64	64.43	Succ	<input type="checkbox"/>
LMO		GEN 503	—	8.23	0	Succ	<input type="checkbox"/>

Positive results with a “wrong” detection of a MP:

Samples: GL 267, GL 275, GL 276, GL283, GL 481, GL 545, GL 562, GL 267 broth stored at 5°C, GL 562 broth stored at 5°C

First example of curve: GL 481



Assay	2	Sample ID	Resi 3	CP 4	TM (°)	In	Status
LMO		GEN 481	+	0	50.83	Succ	<input type="checkbox"/>
LMD		GEN 500	+	22.84	64.43	Succ	<input type="checkbox"/>

Assay	2	Sample ID	Resi 3	CP 4	TM (°)	In	Status
LMO		S23 468	+	0	50	Succ	<input type="checkbox"/>
LMO		S25 537	+	0	49.11	Succ	<input type="checkbox"/>

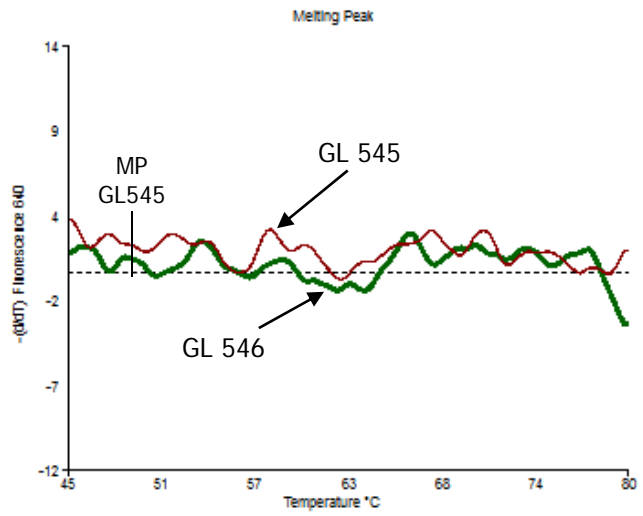
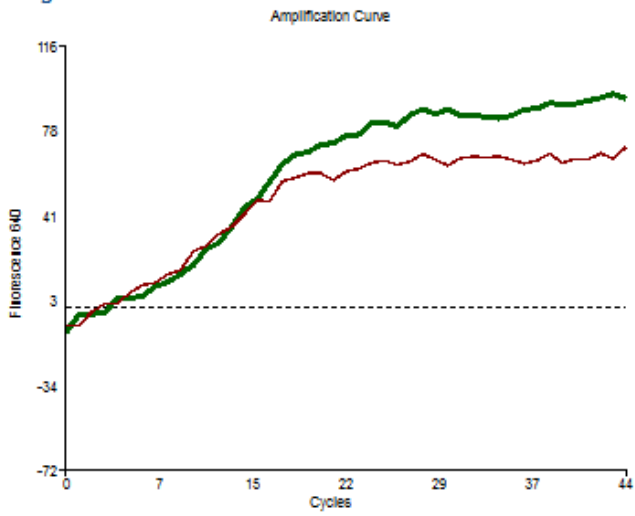
Second example of curve: GL 545

GL 546: negative result

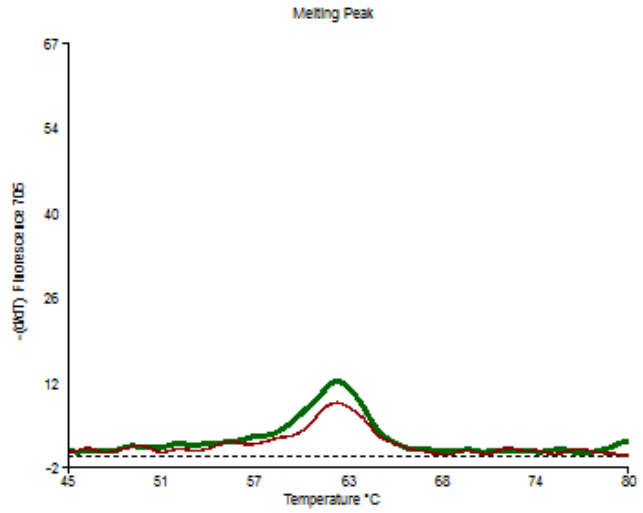
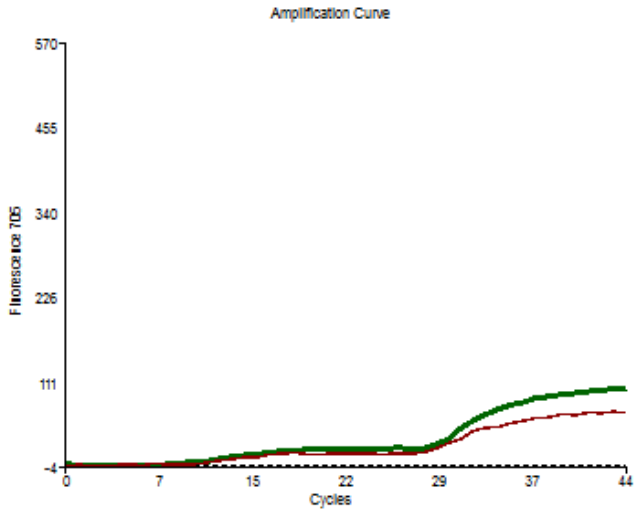
GL 545: false positive result

LMO

Target



Internal Control



Assay	2	Sample ID	Res	3	CP	4	TM (°C)	Ir	Status	M	C	R	C	We	1	Ts	I
LMO		GEN 545	+		0		49	Succ	<input type="checkbox"/>					H7		Positi	4
LMO		GEN 546	-		0		0	Succ	<input type="checkbox"/>					A8		Nega	4

APPENDIX 5

RLOD

RAW RESULTS

Caption:

/ : test not realized

∅ : absence of colonies

FP : false positive result

A : absence

P : presence

0 / 1 / 2 / 3 / 4 : level of typical flora, from absence to high

∅ / L / M / H : level of annex flora, from absence to high

L.m : *Listeria monocytogenes*

Confirmation : streaking on selective medium + ISO 11290-1 confirmation

Conf. 1 : streaking on selective medium + visual reading

Conf. 2 : streaking on selective medium + API Listeria

Conf. 3 : streaking on selective medium + RAPIDEC L-mono

Conf. 4 : streaking on selective medium + Fast Rhamnose

Conf. 5 : streaking on selective medium + ISO 11290-1 confirmation (case n°1)

chromID L. mono: w=white colonies / b=blue colonies

MEAT PRODUCTS

TVC before inoculation : 1.2x10⁴ CFU/g

/

TVC after cold storage: 9.9x10³ CFU/g

Matrix	Contamination level (CFU/25 g)	Sample ID	RM: NF EN ISO 11290-1 (*)						AM: GENE UP										Confirmation on MA samples		Number of positive results per method
			Half Fraser		Fraser		Confirmation	Final result	GENE UP result			Conf. 1		Conf. 2	Conf. 3	Conf. 4	Conf. 5	Final result	Conf. 3	Final result	
			ALOA	PALCAM	ALOA	PALCAM			Result	CP	MP	ALOA	Chrom ID								
Rillettes	/	GLMR01	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 ∅	0 ∅	/	/	/	/	A	-	A	RM = 0/5 AM = 0/5
		GLMR02	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A	
		GLMR03	0 ∅	0 ∅	0 M	0 ∅	/	A	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A	
		GLMR04	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A	
		GLMR05	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A	
	0.6	GLMRL1	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 L	0 ∅	/	/	/	/	A	-	A	RM = 7/20 AM = 8/20
		GLMRL2	0 ∅	0 ∅	0 M	0 M	/	A	-	0,00	0,00	0 L	0 ∅	/	/	/	/	A	-	A	
		GLMRL3	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A	
		GLMRL4	3h+ ∅	2 ∅	4h+ ∅	3 L	+ (L. m)	P	+	22,24	64,39	4h+ ∅	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/	
		GLMRL5	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A	
		GLMRL6	3h+ ∅	3 ∅	3h+ ∅	3 ∅	+ (L. m)	P	+	19,10	64,76	4h+ ∅	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/	
		GLMRL7	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A	
		GLMRL8	0 ∅	0 ∅	0 M	0 M	/	A	+	20,06	64,81	4h+ ∅	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/	
		GLMRL9	3h+ ∅	3 ∅	4h+ ∅	2 M	+ (L. m)	P	+	22,67	64,81	4h+ ∅	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/	
		GLMRL10	0 ∅	0 ∅	0 L	0 M	/	A	+	27,76	64,59	4h+ ∅	3 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/	
		GLMRL11	3h+ ∅	3 ∅	3h+ ∅	4 L	+ (L. m)	P	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A	
		GLMRL12	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A	
		GLMRL13	0 ∅	0 ∅	0 M	0 H	/	A	-	0,00	0,00	0 ∅	0 ∅	/	/	/	/	A	-	A	
		GLMRL14	0 ∅	0 ∅	0 ∅	0 ∅	/	A	+	19,78	64,70	4h+ ∅	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/	
		GLMRL15	0 ∅	0 ∅	0 ∅	0 ∅	/	A	+	24,12	64,81	0 ∅	0 ∅	/	/	/	/	A (FP)	-	A	
GLMRL16	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0 ∅	0 ∅	/	/	/	/	A	-	A			
GLMRL17	2h+ ∅	2 ∅	4h+ ∅	2 M	+ (L. m)	P	-	0,00	0,00	0 ∅	0 ∅	/	/	/	/	A	-	A			
GLMRL18	0 ∅	0 L	0 M	0 M	/	A	+	25,19	64,11	4h+ ∅	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/			
GLMRL19	3h+ ∅	3 ∅	3h+ ∅	2 H	+ (L. m)	P	+	21,24	64,82	4h+ ∅	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/			
GLMRL20	2h+ ∅	3 ∅	3h+ ∅	2 M	+ (L. m)	P	-	0,00	0,00	0 M	0 ∅	/	/	/	/	A	-	A			
1.8	GLMRH1	0 M	0 L	0 ∅	0 ∅	/	A	+	23,42	64,46	4h+ M	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/	RM = 4/5 AM = 5/5	
	GLMRH2	2h+ M	4 ∅	3h+ ∅	2 M	+ (L. m)	P	+	22,67	64,91	4h+ H	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/		
	GLMRH3	3h+ M	4 ∅	4h+ ∅	1 H	+ (L. m)	P	+	19,88	64,98	4h+ H	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/		
	GLMRH4	2h+ M	2 ∅	3h+ ∅	1 H	+ (L. m)	P	+	22,29	64,66	4h+ M	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/		
	GLMRH5	3h+ M	3 ∅	3h+ ∅	4 ∅	+ (L. m)	P	+	22,50	64,55	4h+ H	4 b ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	/	/		

DAIRY PRODUCTS

TVC before inoculation : 2.5x10⁴ CFU/mL

TVC after cold storage: 5.0x10⁵ CFU/mL

Matrix	Contamination level (CFU/25 g)	Sample ID	RM: NF EN ISO 11290-1 (*)						AM: GENE UP										Confirmation on MA samples		Number of positive results per method				
			Half Fraser		Fraser		Confirmation	Final result	GENE UP result			Conf. 1		Conf. 2	Conf. 3	Conf. 4	Conf. 5	Final result	Confirmation	Final result					
			ALOA	PALCAM	ALOA	PALCAM			Result	CP	MP	ALOA	Chrom ID												
Raw milk	/	GLMRM01	0 L	0 L	0 Ø	0 Ø	/	A	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-	A	RM = 0/5 AM = 0/5				
		GLMRM02	0 L	0 L	0 L	0 L	/	A	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-	A					
		GLMRM03	0 Ø	0 L	0 Ø	0 L	/	A	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-	A					
		GLMRM04	0 Ø	0 L	0 L	0 L	/	A	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-	A					
		GLMRM05	0 Ø	0 L	0 L	0 H	/	A	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-	A					
	0.7	GLMRML1	0 Ø	0 Ø	0 Ø	0 L	/	A	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-	A	RM = 7/20 AM = 7/20				
		GLMRML2	1h+ Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	-	0,00	0,00	0 L	0 Ø	/	/	/	/	A	-		A			
		GLMRML3	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0 L	0 Ø	/	/	/	/	A	-	A					
		GLMRML4	0 L	0 Ø	0 H	0 M	/	A	-	0,00	0,00	0 Ø	0 L	/	/	/	/	A	-	A					
		GLMRML5	2h+ Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-		A			
		GLMRML6	1h+ Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	!/-	0,00	0,00	0 L	0 Ø	/	/	/	/	A	-		A			
		GLMRML7	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0 L	0 Ø	/	/	/	/	A	-	A					
		GLMRML8	0 Ø	0 Ø	0 Ø	0 Ø	/	A	+	29,03	65,02	3h+ Ø	3 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+		(L. m)	P	/	/
		GLMRML9	0 Ø	0 L	0 Ø	0 Ø	/	A	+	29,71	65,18	2h+ Ø	2 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+		(L. m)	P	/	/
		GLMRML10	1h+ Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-		A			
		GLMRML11	0 Ø	0 L	0 Ø	0 Ø	/	A	+	29,97	65,28	2h+ Ø	2 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+		(L. m)	P	/	/
		GLMRML12	1h+ Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-		A			
		GLMRML13	0 Ø	0 Ø	0 Ø	0 H	/	A	-	0,00	0,00	0 L	0 Ø	/	/	/	/	A	-	A					
		GLMRML14	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-	A					
		GLMRML15	0 Ø	0 Ø	0 Ø	0 Ø	/	A	+	28,74	65,06	3h+ Ø	3 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+		(L. m)	P	/	/
	GLMRML16	0 Ø	0 L	4h+ Ø	3 Ø	/	A	+	28,82	64,93	3h+ Ø	3 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+	(L. m)	P	/	/		
	GLMRML17	1h+ Ø	1 Ø	3h+ Ø	4 Ø	+	(L. m)	P	-	0,00	0,00	0 L	0 L	/	/	/	/	A	-	A					
	GLMRML18	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0 L	0 Ø	/	/	/	/	A	-	A						
	GLMRML19	0 Ø	0 L	4h+ Ø	4 Ø	+	(L. m)	P	+	0,00	65,75	2h+ Ø	2 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+	(L. m)	P	/	/	
	GLMRML20	0 Ø	0 L	0 Ø	0 Ø	/	A	+	30,22	64,69	3h+ L	2 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+	(L. m)	P	/	/		
2.1	GLMRMH1	0 Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	-	0,00	0,00	0 L	0 Ø	/	/	/	/	A	-	A	RM = 5/5 AM = 4/5				
	GLMRMH2	1h+ Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	+	28,44	65,19	4h+ Ø	3 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+		(L. m)	P	/	/
	GLMRMH3	1h+ Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	+	30,03	65,43	2h+ Ø	2 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+		(L. m)	P	/	/
	GLMRMH4	1h+ Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	+	29,02	65,08	3h+ Ø	3 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+		(L. m)	P	/	/
	GLMRMH5	2h+ Ø	1 Ø	4h+ Ø	4 Ø	+	(L. m)	P	+	29,10	65,32	3h+ Ø	2 b Ø	+	(L. m)	+	(L. m)	+	(L. m)	+		(L. m)	P	/	/

Salmon offcuts

#	Matrix	Contamination level (CFU/25 g)	Sample ID	RM: NF EN ISO 11290-1 (*)						AM: GENE UP									Confirmation on MA		Number of positive results per method
				Half Fraser		Fraser		Confir- mation	Final result	GENE UP result			Conf. 1 ALOA	Conf. 2	Conf. 3	Conf. 4	Conf. 5	Final result	Confir- mation	Final result	
				ALOA	PALCAM	ALOA	PALCAM			Result	CP	MP									
1	Salmon offcuts	0	GLMSE1	0 Ø	0 Ø	0 L	0 H	/	A	-	0,00	0,00	0 M	/	/	/	/	A	-	A	RM = 0/5 AM = 0/5
2			GLMSE2	0 Ø	0 Ø	0 L	0 L	/	A	-	0,00	0,00	0 M	/	/	/	/	A	-	A	
3			GLMSE3	0 Ø	0 Ø	0 M	0 H	/	A	-	0,00	0,00	0 L	/	/	/	/	A	-	A	
4			GLMSE4	0 Ø	0 L	0 L	0 M	/	A	-	0,00	0,00	0 M	/	/	/	/	A	-	A	
5			GLMSE5	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0 M	/	/	/	/	A	-	A	
1		0.9	GLMSE6	2h+ L	2L	3h+ L	2M	+(L.m)	P	-	0,00	0,00	0L	/	/	/	/	A	-	A	RM = 16/20 AM = 13/20
2			GLMSE7	2h+ L	2L	3h+ L	2M	+(L.m)	P	-	0,00	0,00	0M	/	/	/	/	A	-	A	
3			GLMSE8	3h+ L	2M	3h+ L	2H	+(L.m)	P	+	22,98	53,18	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
4			GLMSE9	0 L	0L	0L	0L	/	A	+	26,16	52,54	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
5			GLMSE10	1h+ Ø	1L	3h+Ø	3L	+(L.m)	P	-	0,00	0,00	0L	/	/	/	/	A	-	A	
6			GLMSE11	1h+ M	1L	3h+ L	3H	+(L.m)	P	+	28,93	52,53	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
7			GLMSE12	1h+ L	1L	3h+ Ø	3L	+(L.m)	P	+	26,98	52,42	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
8			GLMSE13	1h+ Ø	1M	3h+Ø	3L	+(L.m)	P	-	0,00	0,00	0Ø	/	/	/	/	A	-	A	
9			GLMSE14	2h+ L	2L	3h+ Ø	3L	+(L.m)	P	+	27,05	52,59	3h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
10			GLMSE15	2h+ Ø	2L	3h+ Ø	3L	+(L.m)	P	+	26,57	52,55	3h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
11			GLMSE16	0L	0M	0H	0H	/	A	+	27,71	52,61	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
12			GLMSE17	0L	0M	0H	0H	/	A	+	22,87	53,50	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
13			GLMSE18	2h+ Ø	2L	3h Ø	3M	+(L.m)	P	+	27,55	52,70	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
14			GLMSE19	0L	0H	0H	0H	/	A	-	0,00	0,00	0Ø	/	/	/	/	A	-	A	
15			GLMSE20	2h+ Ø	2L	3h+ Ø	3M	+(L.m)	P	+	26,03	52,48	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
16			GLMSE21	1h+ L	1H	3h+ Ø	3L	+(L.m)	P	+	24,50	52,68	3h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
17			GLMSE22	3h+ L	2M	3h+ L	3M	+(L.m)	P	+	23,65	52,76	3h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
18			GLMSE23	3h+ Ø	3L	3h+ Ø	3M	+(L.m)	P	+	25,24	52,72	3h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
19			GLMSE24	3h+ L	3H	3h+ L	2H	+(L.m)	P	-	0,00	0,00	0Ø	/	/	/	/	A	-	A	
20			GLMSE25	3h+ L	3L	3h+ L	3H	+(L.m)	P	-	0,00	0,00	0Ø	/	/	/	/	A	-	A	
1		2.8	GLMSE26	3h+ Ø	3L	3h+ Ø	3L	+(L.m)	P	+	24,93	52,70	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	RM = 5/5 AM = 5/5
2			GLMSE27	2h+ L	2L	3h+ Ø	3L	+(L.m)	P	+	24,01	52,78	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
3			GLMSE28	4h+ Ø	3L	3h+ Ø	3L	+(L.m)	P	+	24,85	52,62	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
4			GLMSE29	3h+ Ø	3L	3h+ Ø	3L	+(L.m)	P	+	22,72	52,67	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
5			GLMSE30	3h+ Ø	3L	3h+ Ø	3L	+(L.m)	P	+	23,61	52,79	4h+ Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
Total viable count : 5.4x10 ² CFU / g																					

Mix of precooked vegetables

#	Sample	Contamination level (CFU/25 g)	Sample ID	RM: NF EN ISO 11290-1 (*)					AM: GENE UP							Confirmation on MA samples		Number of positive results per method	
				Half Fraser		Fraser		Confir- mation	Final result	GENE UP result	Conf. 1 ALOA	Conf. 2	Conf. 3	Conf. 4	Conf. 5	Final result	Confirmation		Final result
				ALOA	PALCAM	ALOA	PALCAM												
1	Mix of precooked vegetables	0	GLMV01	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	RM = 0/5 AM = 0/5
2			GLMV02	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	
3			GLMV03	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	
4			GLMV04	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	
5			GLMV05	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	
6		0.5	GLMV06	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	RM = 6/20 AM = 11/20
7			GLMV07	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	
8			GLMV08	3h+ ∅	3 ∅	4h+ ∅	4 ∅	+ (L. m)	P	+	3h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
9			GLMV09	3h+ ∅	3 ∅	4h+ ∅	4 ∅	+ (L. m)	P	+	4h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
10			GLMV10	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	
11			GLMV11	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	
12			GLMV12	0 ∅	0 ∅	0 ∅	0 ∅	/	A	+	3h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
13			GLMV13	0 ∅	0 ∅	0 ∅	0 ∅	/	A	+	3h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
14			GLMV14	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	
15			GLMV15	0 ∅	0 ∅	0 ∅	0 ∅	/	A	+	3h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
16			GLMV16	0 L	0 L	0 ∅	0 ∅	/	A	+	4h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
17			GLMV17	3h+ ∅	3 ∅	4h+ ∅	4 ∅	+ (L. m)	P	+	4h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
18			GLMV18	3h+ ∅	3 ∅	4h+ ∅	4 ∅	+ (L. m)	P	-	0 ∅	/	/	/	/	A	-	A	
19			GLMV19	3h+ ∅	3 ∅	1h-4h+ ∅	4 ∅	+ (L. m)	P	+	4h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
20			GLMV20	0 ∅	0 ∅	0 ∅	0 ∅	/	A	+	3h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
21			GLMV21	3h+ ∅	3 ∅	4h+ ∅	4 ∅	+ (L. m)	P	-	0 ∅	/	/	/	/	A	-	A	
22			GLMV22	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0 ∅	/	/	/	/	A	-	A	
23			GLMV23	0 ∅	0 ∅	0 ∅	0 ∅	/	A	+	0 ∅	/	/	/	/	A (FP)	-	A	
24			GLMV24	0 ∅	0 ∅	0 ∅	0 ∅	/	A	+	4h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
25			GLMV25	0 ∅	0 L	0 L	0 M	/	A	+	4h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
26		2.3	GLMV26	3h+ ∅	4 ∅	4h+ ∅	4 ∅	+ (L. m)	P	+	4h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	RM = 4/5 AM = 4/5
27			GLMV27	3h+ ∅	3 ∅	1h-4h+ ∅	4 ∅	+ (L. m)	P	-	0 ∅	/	/	/	/	A	-	A	
28			GLMV28	0 ∅	0 ∅	0 ∅	0 ∅	/	A	+	4h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
29			GLMV29	3h+ ∅	3 ∅	4h+ ∅	4 ∅	+ (L. m)	P	+	3h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
30			GLMV30	3h+ ∅	3 ∅	4h+ ∅	4 ∅	+ (L. m)	P	+	4h+ ∅	+ (L. m)	+ (L. m)	+ (L. m)	+ (L. m)	P	+ (L. m)	P	
Total viable count : <4.0x10 ¹ CFU/g																			

Mixed salad

#	Matrix	Contamination level (CFU/25 g)	Sample ID	RM: NF EN ISO 11290-1 (*)					AM: GENE UP							Confirmation on MA samples		Number of positive results per method	
				Half Fraser		Fraser		Confir- mation	Final result	GENE UP result	Conf. 1 ALOA	Conf. 2	Conf. 3	Conf. 4	Conf. 5	Final result	Confirmation		Final result
				ALOA	PALCAM	ALOA	PALCAM												
1	Mixed salad	0	GLMSE1	0 Ø	0 Ø	0 L	0 H	/	A	-	0 M	/	/	/	/	A	-	A	RM = 0/5 AM = 0/5
2			GLMSE2	0 Ø	0 Ø	0 L	0 L	/	A	-	0 M	/	/	/	/	A	-	A	
3			GLMSE3	0 Ø	0 Ø	0 M	0 H	/	A	-	0 L	/	/	/	/	A	-	A	
4			GLMSE4	0 Ø	0 L	0 L	0 M	/	A	-	0 M	/	/	/	/	A	-	A	
5			GLMSE5	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0 M	/	/	/	/	A	-	A	
6		0,9	GLMSE6	1h+ Ø	2 L	3h+ Ø	3 M	+(L. m)	P	+	4h+ L	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	RM = 13/20 AM = 15/20
7			GLMSE7	0 Ø	0 L	0 H	0 H	/	A	-	0 Ø	/	/	/	/	A	-	A	
8			GLMSE8	2h+ Ø	2 Ø	3h+ Ø	4 Ø	+(L. m)	P	+	3h+ L	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
9			GLMSE9	1h+ Ø	1 Ø	3h+ Ø	3 Ø	+(L. m)	P	+	4h+ Ø	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
10			GLMSE10	0 Ø	0 Ø	0 M	0 H	/	A	-	0 L	/	/	/	/	A	-	A	
11			GLMSE11	1h+ Ø	1 Ø	3h+ L	4 L	+(L. m)	P	-	0 L	/	/	/	/	A	-	A	
12			GLMSE12	1h+ Ø	1 Ø	3h+ Ø	3 M	+(L. m)	P	+	4h+ M	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
13			GLMSE13	1h+ Ø	1 Ø	4h+ Ø	4 Ø	+(L. m)	P	+	3h+ M	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
14			GLMSE14	0 Ø	0 L	0 Ø	0 Ø	/	A	+	2h+ H	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
15			GLMSE15	1h+ Ø	1 L	3h+ Ø	4 L	+(L. m)	P	+	4h+ L	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
16			GLMSE16	2h+ Ø	2 L	3h+ Ø	2 M	+(L. m)	P	+	3h+ L	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
17			GLMSE17	2h+ Ø	2 L	3h+ Ø	4 L	+(L. m)	P	+	3h+ M	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
18			GLMSE18	0 Ø	0 L	0 L	0 H	/	A	+	3h+ M	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
19			GLMSE19	0 Ø	0 L	0 L	0 H	/	A	+	3h+ H	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
20			GLMSE20	1h+ Ø	1 L	3h+ Ø	4 L	+(L. m)	P	+	3h+ M	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
21			GLMSE21	2h+ L	2 L	3h+ Ø	4 Ø	+(L. m)	P	+	1h+ M	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
22			GLMSE22	0 Ø	0 L	0 L	0 H	/	A	+	0 M	/	/	/	/	A (FP)	-	A (FP)	
23			GLMSE23	1h+ Ø	1 L	4h+ Ø	4 L	+(L. m)	P	+	3h+ H	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
24			GLMSE24	1h+ Ø	1 L	4h+ Ø	4 Ø	+(L. m)	P	+	1h+ H	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
25			GLMSE25	0 Ø	0 L	0 Ø	0 Ø	/	A	-	0 M	/	/	/	/	A	-	A	
26		2,7	GLMSE26	2h+ Ø	1 L	3h+ Ø	3 L	+(L. m)	P	+	4h+ L	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	RM = 4/5 AM = 5/5
27			GLMSE27	2h+ Ø	2 L	4h+ Ø	4 Ø	+(L. m)	P	+	3h+ M	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
28			GLMSE28	2h+ Ø	2 L	4h+ Ø	4 L	+(L. m)	P	+	4h+ L	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
29			GLMSE29	0 Ø	0 L	0 L	0 L	/	A	+	4h+ L	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
30			GLMSE30	2h+ L	3 L	3h+ Ø	4 L	+(L. m)	P	+	1h+ M	+(L. m)	+(L. m)	+(L. m)	+(L. m)	P	+(L. m)	P	
Total viable count : 7.4x10 ² CFU/g																			

Process water

#	Matrix	Contamination level (CFU/25 g)	Sample ID	RM: NF EN ISO 11290-1 (*)						AM: GENE UP									Confirmation on MA		Number of positive results per method
				Half Fraser		Fraser		Confir- mation	Final result	GENE UP result			Conf. 1	Conf. 2	Conf. 3	Conf. 4	Conf. 5	Final result	Confir- mation	Final result	
				ALOA	PALCAM	ALOA	PALCAM			Result	CP	MP									
1	Process water	0	GLMPW1	0 Ø	0 Ø	0 L	0 H	/	A	-	0,00	0,00	0 M	/	/	/	/	A	-	A	RM = 0/5 AM = 0/5
2			GLMPW2	0 Ø	0 Ø	0 L	0 L	/	A	-	0,00	0,00	0 L	/	/	/	/	A	-	A	
3			GLMPW3	0 Ø	0 Ø	0 M	0 H	/	A	-	0,00	0,00	0 L	/	/	/	/	A	-	A	
4			GLMPW4	0 Ø	0 L	0 L	0 M	/	A	-	0,00	0,00	0 M	/	/	/	/	A	-	A	
5			GLMPW5	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0 L	/	/	/	/	A	-	A	
1		1.6	GLMPW6	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0L	/	/	/	/	A	-	A	RM = 6/20 AM = 8/20
2			GLMPW7	0 Ø	0 Ø	0 Ø	0 Ø	/	A	+	17,67	65,17	4h+Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
3			GLMPW8	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0L	/	/	/	/	A	-	A	
4			GLMPW9	0 Ø	0 Ø	0 Ø	0 Ø	/	A	+	18,82	65,28	4h+Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
5			GLMPW10	3h+Ø	3Ø	4h+Ø	4Ø	+(L.m)	P	-	0,00	0,00	0M	/	/	/	/	A	-	A	
6			GLMPW11	0Ø	0Ø	0Ø	0Ø	/	A	-	0,00	0,00	0M	/	/	/	/	A	-	A	
7			GLMPW12	4h+Ø	4Ø	4h+Ø	4Ø	+(L.m)	P	-	0,00	0,00	0M	/	/	/	/	A	-	A	
8			GLMPW13	0Ø	0Ø	0Ø	0Ø	/	A	-	0,00	0,00	0L	/	/	/	/	A	-	A	
9			GLMPW14	3h+Ø	3Ø	3h+Ø	3Ø	+(L.m)	P	+	16,88	65,42	4h+Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
10			GLMPW15	0Ø	0Ø	0Ø	0Ø	/	A	-	0,00	0,00	0L	/	/	/	/	A	-	A	
11			GLMPW16	3h+Ø	3Ø	3h+Ø	3Ø	+(L.m)	P	+	20,21	65,19	4h+Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
12			GLMPW17	0 Ø	0 Ø	0 Ø	0 Ø	/	A	+	17,68	65,19	4h+Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
13			GLMPW18	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0M	/	/	/	/	A	-	A	
14			GLMPW19	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0M	/	/	/	/	A	-	A	
15			GLMPW20	3h+Ø	3Ø	3h+Ø	4Ø	+(L.m)	P	+	24,03	65,07	3h+L	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
16			GLMPW21	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0L	/	/	/	/	A	-	A	
17			GLMPW22	3h+Ø	3Ø	0Ø	3Ø	+(L.m)	P	-	0,00	0,00	0M	/	/	/	/	A	-	A	
18			GLMPW23	0 Ø	0 Ø	0 Ø	0 Ø	/	A	+	21,04	65,14	4h+Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
19			GLMPW24	0 Ø	0 Ø	0 Ø	0 Ø	/	A	+	16,53	65,34	4h+Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
20			GLMPW25	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0H	/	/	/	/	A	-	A	
1		4.4	GLMPW26	3h+ Ø	4Ø	3h+ Ø	4Ø	+(L.m)	P	-	0,00	0,00	0L	/	/	/	/	A	-	A	RM = 2/5 AM = 2/5
2			GLMPW27	0 Ø	0 Ø	0 Ø	0 Ø	/	A	-	0,00	0,00	0M	/	/	/	/	A	-	A	
3			GLMPW28	3h+Ø	4Ø	2h+Ø	0Ø	+(L.m)	P	+	24,89	65,20	3h+Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
4			GLMPW29	0Ø	0Ø	0Ø	0Ø	/	A	+	20,94	62,08	4h+Ø	+(L.m)	+(L.m)	+(L.m)	+(L.m)	P	+(L.m)	P	
5			GLMPW30	0Ø	0Ø	0Ø	0Ø	/	A	-	0,00	0,00	0M	/	/	/	/	A	-	A	
Total viable count : 3.0x10 ² CFU / mL																					

Swab

#	Matrix	Contamination level (CFU/25 g)	Sample ID	RM: NF EN ISO 11290-1 (*)						AM: GENE UP							Confirmation on MA		Number of positive results per method						
				Half Fraser		Fraser		Confir- mation	Final result	GENE UP result			Conf. 1	Conf. 2	Conf. 3	Final result	Confir- mation	Final result							
				ALOA	PALCAM	ALOA	PALCAM			Result	CP	MP								ALOA					
1	Swab	0	GLMS1	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0∅	/	/	A	-	A	RM = 0/5 AM = 0/5						
2			GLMS2	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0∅	/	/	A	-	A							
3			GLMS3	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0∅	/	/	A	-	A							
4			GLMS4	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0∅	/	/	A	-	A							
5			GLMS5	0 ∅	0 ∅	0 ∅	0 ∅	/	A	-	0,00	0,00	0∅	/	/	A	-	A							
1		1.1	GLMS6	3h+∅	3∅	3h+∅	3∅	+	(L.m)	P	+	25,93	65,34	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P	RM = 13/20 AM = 12/20		
2			GLMS7	2h+∅	3∅	2h+∅	3∅	+	(L.m)	P	+	25,62	65,24	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P			
3			GLMS8	2h+∅	2∅	2h+∅	4∅	+	(L.m)	P	-	7,56	0,00	0∅	/	/	A	-	A						
4			GLMS9	3h+∅	2∅	3h+∅	3∅	+	(L.m)	P	+	23,18	65,20	2h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P			
5			GLMS10	0∅	0∅	0∅	0∅	/	A	-	0,00	0,00	0∅	/	/	A	-	A							
6			GLMS11	0∅	0∅	0∅	0∅	/	A	-	0,00	0,00	0∅	/	/	A	-	A							
7			GLMS12	3h+∅	3∅	3h+∅	4∅	+	(L.m)	P	+	25,89	65,11	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P			
8			GLMS13	2h+∅	2∅	2h+∅	3∅	+	(L.m)	P	-	0,00	0,00	0∅	/	/	A	-	A						
9			GLMS14	0∅	0∅	∅	0∅	/	A	-	0,00	0,00	0∅	/	/	A	-	A							
10			GLMS15	3h+∅	3∅	2h+∅	4∅	+	(L.m)	P	-	0,00	0,00	0∅	/	/	A	-	A						
11			GLMS16	2h+∅	2∅	2h+∅	3∅	+	(L.m)	P	+	25,94	65,35	2h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P			
12			GLMS17	0∅	0∅	0∅	0∅	/	A	-	0,00	0,00	0∅	/	/	A	-	A							
13			GLMS18	3h+∅	3∅	3h+∅	3∅	+	(L.m)	P	+	27,72	65,64	2h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P			
14			GLMS19	3h+∅	3∅	4h	4∅	+	(L.m)	P	+	26,06	65,34	2h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P			
15			GLMS20	0∅	0∅	0∅	0∅	/	A	+	24,19	65,11	2h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P				
16			GLMS21	3h+∅	3∅	3h+∅	3∅	+	(L.m)	P	+	25,04	65,47	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P			
17			GLMS22	0∅	0∅	0∅	0∅	/	A	+	25,08	65,28	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P				
18			GLMS23	3h+∅	3∅	3h+∅	3∅	+	(L.m)	P	-	0,00	0,00	0∅	/	/	A	-	A						
19			GLMS24	2h+∅	3∅	3h+∅	3∅	+	(L.m)	P	+	24,54	65,34	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P			
20			GLMS25	0∅	0∅	0∅	0∅	/	A	+	25,66	65,24	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)	P				
1			3.1	GLMS26	3h+∅	3∅	3h+∅	4∅	+	(L.m)	P	+	23,02	65,20	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)		P	RM = 5/5 AM = 5/5
2				GLMS27	2h+∅	2∅	2h+∅	3∅	+	(L.m)	P	+	23,59	65,21	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)		P	
3				GLMS28	3h+∅	3∅	3h+∅	3∅	+	(L.m)	P	+	22,97	65,19	2h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)		P	
4				GLMS29	3h+∅	3∅	3h+∅	3∅	+	(L.m)	P	+	23,45	65,38	2h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)		P	
5				GLMS30	3h+∅	3∅	3h+∅	3∅	+	(L.m)	P	+	22,15	65,48	3h+∅	+	(L.m)	+	(L.m)	P	+	(L. m)		P	
Total viable count : <10 CFU / g																									

APPENDIX 6

INCLUSIVITY AND EXCLUSIVITY

INCLUSIVITY

Number	Code	Microorganism	Origin	Gene-UP result			Confirmation
				CP	MP	Result	
1	LIS.4.1	<i>Listeria monocytogenes</i>	CIP 78.31	19.17	64.74	+	Positive
2	LIS.4.2	<i>Listeria monocytogenes</i>	Clinical environment	19.34	61.95	+	Positive
3	LIS.4.4	<i>Listeria monocytogenes 1/2a</i>	zucchini cheese brochette	21.64	64.97	+	Positive
4	LIS.4.5	<i>Listeria monocytogenes 1/2a</i>	ham and vegetables	18.01	64.73	+	Positive
5	LIS.4.6	<i>Listeria monocytogenes 1/2a</i>	ham cheese sandwich	20.76	65.03	+	Positive
6	LIS.4.7	<i>Listeria monocytogenes 1/2a</i>	ham cheese sandwich	21.14	62.19	+	Positive
7	LIS.4.8	<i>Listeria monocytogenes 1/2a</i>	tuna egg surimi sandwich	19.49	64.72	+	Positive
8	LIS.4.9	<i>Listeria monocytogenes 1/2a</i>	roasted beef bone meal	18.92	64.94	+	Positive
9	LIS.4.10	<i>Listeria monocytogenes 1/2a</i>	salad	19.00	62.02	+	Positive
10	LIS.4.11	<i>Listeria monocytogenes 1/2a</i>	curry chicken	20.13	61.96	+	Positive
11	LIS.4.12	<i>Listeria monocytogenes 1/2a</i>	smoked salmon	18.81	64.79	+	Positive
12	LIS.4.13	<i>Listeria monocytogenes 1/2a</i>	foie gras	19.15	64.88	+	Positive
13	LIS.4.14	<i>Listeria monocytogenes 1/2a</i>	kipiti sauce	19.80	62.03	+	Positive
14	LIS.4.15	<i>Listeria monocytogenes 1/2a</i>	salmon tartare	19.22	64.84	+	Positive
15	LIS.4.16	<i>Listeria monocytogenes 1/2a</i>	swab	19.44	64.74	+	Positive
16	LIS.4.17	<i>Listeria monocytogenes 1/2a</i>	raw vegetables	19.29	64.91	+	Positive
17	LIS.4.18	<i>Listeria monocytogenes 1/2a</i>	vegetables salad	21.04	61.80	+	Positive
18	LIS.4.19	<i>Listeria monocytogenes 1/2a</i>	Guinea fowl	22.87	64.54	+	Positive
19	LIS.4.20	<i>Listeria monocytogenes 1/2a</i>	bacon vegetables sandwich	18.66	64.54	+	Positive
20	LIS.4.21	<i>Listeria monocytogenes 1/2a</i>	CIP 103574	19.48	64.94	+	Positive
21	LIS.4.22	<i>Listeria monocytogenes 1/2a</i>	CIP 104794	25.32	62.08	+	Positive
22	LIS.4.23	<i>Listeria monocytogenes 1/2a</i>	Cheese	16.70	64.96	+	Positive
23	LIS.4.24	<i>Listeria monocytogenes 1/2a</i>	ready to eat meal with cheese	22.74	64.71	+	Positive
24	LIS.4.25	<i>Listeria monocytogenes 1/2a</i>	Fish and vegetables provençale	18.01	65.13	+	Positive
25	LIS.4.26	<i>Listeria monocytogenes 1/2a</i>	Ham	19.17	64.93	+	Positive
26	LIS.4.27	<i>Listeria monocytogenes 1/2a</i>	Minced meat	17.13	65.09	+	Positive
27	LIS.4.28	<i>Listeria monocytogenes 1/2b</i>	Duck	18.65	64.78	+	Positive
28	LIS.4.29	<i>Listeria monocytogenes 1/2b</i>	Praliné	20.56	64.89	+	Positive
29	LIS.4.30	<i>Listeria monocytogenes 1/2b</i>	Raw turkey	18.50	64.84	+	Positive
30	LIS.4.31	<i>Listeria monocytogenes 1/2b</i>	Rollmops	17.51	65.09	+	Positive
31	LIS.4.32	<i>Listeria monocytogenes 1/2b</i>	Raw milk	20.48	64.75	+	Positive
32	LIS.4.33	<i>Listeria monocytogenes 1/2c</i>	Minced meat	17.33	64.70	+	Positive
33	LIS.4.34	<i>Listeria monocytogenes 1/2c</i>	Gouda	19.35	64.35	+	Positive
34	LIS.4.35	<i>Listeria monocytogenes 1/2c</i>	« chef » salad sandwich	19.93	62.38	+	Positive
35	LIS.4.36	<i>Listeria monocytogenes 1/2c</i>	CIP 103573	21.22	62.25	+	Positive
36	LIS.4.37	<i>Listeria monocytogenes 1/2c</i>	Duck foie gras	19.20	62.31	+	Positive
37	LIS.4.38	<i>Listeria monocytogenes 1/2c</i>	Duck foie gras	20.66	62.32	+	Positive
38	LIS.4.39	<i>Listeria monocytogenes 1/2c</i>	Salmon tartare	20.11	62.29	+	Positive
39	LIS.4.40	<i>Listeria monocytogenes 1/2c</i>	Ktipiti sauce	20.08	62.08	+	Positive
40	LIS.4.41	<i>Listeria monocytogenes 1/2c</i>	Foie gras	21.01	62.10	+	Positive
41	LIS.4.42	<i>Listeria monocytogenes 3a</i>	Smoked salmon	17.06	64.82	+	Positive
42	LIS.4.43	<i>Listeria monocytogenes 3a</i>	Sliced bacon	17.48	64.86	+	Positive
43	LIS.4.44	<i>Listeria monocytogenes 3a</i>	Swab	19.46	64.97	+	Positive
44	LIS.4.45	<i>Listeria monocytogenes 3a</i>	Roasted bacon	20.51	65.14	+	Positive
45	LIS.4.46	<i>Listeria monocytogenes 3a</i>	Goat cheese sandwich	19.87	65.02	+	Positive
46	LIS.4.47	<i>Listeria monocytogenes 4b</i>	Salmon slices	19.62	64.98	+	Positive
47	LIS.4.48	<i>Listeria monocytogenes 4b</i>	CIP 103575	21.73	64.77	+	Positive
48	LIS.4.49	<i>Listeria monocytogenes 4b</i>	CIP 7838	20.83	64.86	+	Positive
49	LIS.4.50	<i>Listeria monocytogenes 4b</i>	Salmon swab	19.76	65.17	+	Positive
50	LIS.4.51	<i>Listeria monocytogenes 4c</i>	CIP 7839	17.21	64.46	+	Positive

EXCLUSIVITY

Number	Code	Microorganism	Origin	Gene-UP result		
				CP	MP	Result
1	LIS.1.1	<i>Listeria grayi</i>	CIP 105447T	0,00	0,00	-
2	LIS.2.1	<i>Listeria innocua</i>	Vegetables sandwich	0,00	0,00	-
3	LIS.2.2	<i>Listeria innocua</i>	Bacon vegetables sandwich	0,00	0,00	-
4	LIS.2.3	<i>Listeria innocua</i>	Door swab	0,00	0,00	-
5	LIS.2.4	<i>Listeria innocua</i>	CIP 80.12	0,00	0,00	-
6	LIS.2.5	<i>Listeria innocua</i>	CTSCCV	0,00	0,00	-
7	LIS.2.6	<i>Listeria innocua</i>	Pork	0,00	0,00	-
8	LIS.2.7	<i>Listeria innocua</i>	Chicken bacon sandwich	0,00	0,00	-
9	LIS.2.8	<i>Listeria innocua</i>	Beef tongue	0,00	0,00	-
10	LIS.2.9	<i>Listeria innocua</i>	Beef meat	0,00	0,00	-
11	LIS.2.10	<i>Listeria innocua</i>	CIP 80.11	0,00	0,00	-
12	LIS.3.1	<i>Listeria ivanovii</i>	Raw milk	0,00	0,00	-
13	LIS.3.2	<i>Listeria ivanovii</i>	CTSCCV	0,00	0,00	-
14	LIS.3.4	<i>Listeria ivanovii subsp. Londoniensis</i>	CIP 103505	0,00	0,00	-
15	LIS.5.1	<i>Listeria seeligeri</i>	CIP 79.46	0,00	0,00	-
16	LIS.5.2	<i>Listeria seeligeri</i>	CTSCCV	0,00	0,00	-
17	LIS.6.1	<i>Listeria welshimeri</i>	CIP 81.48	0,00	0,00	-
18	LIS.6.2	<i>Listeria welshimeri</i>	CIP 81.94 T	0,00	0,00	-
19	LIS.6.3	<i>Listeria welshimeri</i>	CTSCCV	0,00	0,00	-
20	BAC.1.1	<i>Bacillus cereus</i>	Dairy industry	0,00	0,00	-
21	BAC.2.1	<i>Bacillus circulans</i>	Dairy industry	0,00	0,00	-
22	BAC.4.1	<i>Bacillus subtilis</i>	Pudding	0,00	0,00	-
23	BRE.1.1	<i>Brevibacterium casei</i>	Dairy product	0,00	0,00	-
24	ENTC.1.2	<i>Enterococcus faecalis</i>	ATCC 33186	0,00	0,00	-
25	ENTC.2.1	<i>Enterococcus faecium</i>	Dairy industry	0,00	0,00	-
26	LACB.1.1	<i>Lactobacillus casei</i>	Dairy product	0,00	0,00	-
27	LACB.3.1	<i>Lactobacillus leishmanii</i>	CIP 53.61	0,00	0,00	-
28	MIC.1.1	<i>Micrococcus luteus</i>	Dairy industry	0,00	0,00	-
29	RHO.1.1	<i>Rhodococcus equi</i>	CIP 58.69	0,00	0,00	-
30	STA.2.1	<i>Staphylococcus epidermidis</i>	Dairy product	0,00	0,00	-

APPENDIX 7

**RESULTS OF THE EXPERT LABORATORY
INTERLABORATORY STUDY OF APRIL**

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		ISHA	Aspect colonies	Confirmation
ISHA	CP	MP	Result	CP	MP	Result						
2	0,00	0,00	-	/	/	/	-	A	2	-	/	A
4	0,00	0,00	-	/	/	/	-	A	4	-	/	A
7	0,00	0,00	-	/	/	/	-	A	7	-	/	A
9	0,00	0,00	-	/	/	/	-	A	9	-	/	A
15	0,00	0,00	-	/	/	/	-	A	15	-	/	A
17	0,00	0,00	-	/	/	/	-	A	17	-	/	A
18	0,00	0,00	-	/	/	/	-	A	18	-	/	A
24	0,00	0,00	-	/	/	/	-	A	24	-	/	A
3	0,00	0,00	-	/	/	/	-	A	3	-	/	A
5	0,00	0,00	-	/	/	/	-	A	5	-	/	A
8	0,00	0,00	-	/	/	/	-	A	8	-	/	A
13	0,00	0,00	-	/	/	/	-	A	13	-	/	A
16	0,00	0,00	-	/	/	/	-	A	16	-	/	A
20	26,88	64,82	+	/	/	/	+	P	20	-	/	A
22	24,19	64,58	+	/	/	/	+	P	22	-	/	A
23	20,92	64,81	+	/	/	/	+	P	23	+	+	P
1	17,98	64,82	+	/	/	/	+	P	1	+	+	P
6	19,05	64,76	+	/	/	/	+	P	6	+	+	P
10	17,96	64,63	+	/	/	/	+	P	10	+	+	P
11	18,06	64,80	+	/	/	/	+	P	11	+	+	P
12	18,51	64,87	+	/	/	/	+	P	12	+	+	P
14	17,96	64,76	+	/	/	/	+	P	14	+	+	P
19	19,06	64,78	+	/	/	/	+	P	19	+	+	P
21	18,42	64,79	+	/	/	/	+	P	21	+	+	P

Incubation time of the alternative method broth :	
2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	NA
5	NA
8	NA
13	NA
16	NA
20	PD
22	PD
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

22h

APPENDIX 8

**RESULTS OF THE COLLABORATORS
INTERLABORATORY STUDY OF APRIL**

A=absence, P=presence, +=test positive, -=test negative, /=test not realized

Code	Alternative method								Code	Reference method			
	GENE-UP L. monocytogenes									ISO 11290-1/A1			
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		A	Colonies aspect	Confirmation	Final result
CP	MP	Result	CP	MP	Result								
2	0,00	0,00	-	0,00	0,00	-	-	-	A	2	-	/	A
4	8,19	0,00	-	0,00	0,00	-	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	-	A	7	-	/	A
9	0,00	0,00	-	0,00	0,00	-	-	-	A	9	-	/	A
15	0,00	51,33	+	0,00	0,00	-	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	-	A	17	-	/	A
18	0,00	0,00	-	0,00	0,00	-	-	-	A	18	-	/	A
24	0,00	50,83	+	0,00	0,00	-	-	-	A	24	-	/	A
3	24,96	65,80	+	24,50	65,99	+	+	+	P	3	+	+	P
5	23,98	65,82	+	23,29	65,69	+	+	+	P	5	+	+	P
8	21,89	66,11	+	22,78	65,99	+	+	+	P	8	+	+	P
13	22,66	65,82	+	21,96	65,64	+	+	+	P	13	+	+	P
16	22,81	66,11	+	23,22	65,92	+	+	+	P	16	+	+	P
20	22,31	66,29	+	19,94	65,81	+	+	+	P	20	+	+	P
22	19,76	65,86	+	19,19	65,79	+	+	+	P	22	+	+	P
23	20,96	65,77	+	20,32	65,78	+	+	+	P	23	+	+	P
1	18,00	65,82	+	18,72	66,20	+	+	+	P	1	+	+	P
6	17,52	65,88	+	17,49	65,83	+	+	+	P	6	+	+	P
10	18,49	65,90	+	17,84	65,59	+	+	+	P	10	+	+	P
11	18,24	65,91	+	17,66	65,75	+	+	+	P	11	+	+	P
12	19,27	65,54	+	18,49	65,71	+	+	+	P	12	+	+	P
14	19,29	65,95	+	18,94	65,89	+	+	+	P	14	+	+	P
19	18,08	65,70	+	17,48	65,94	+	+	+	P	19	+	+	P
21	17,70	65,83	+	17,31	65,59	+	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 24h

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	PA
13	PA
16	PA
20	PA
22	PA
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence			
	GENE-UP L. monocytogenes									ISO 11290-1/A1			
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		B	Colonies aspect	Confir-mation	Résultat final
CP	MP	Result	CP	MP	Result								
2	0,00	50,00	+	0,00	0,00	-	-	-	A	2	-	/	A
4	0,00	49,00	+	0,00	0,00	-	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	-	A	7	-	/	A
9	10,00	0,00	-	0,00	0,00	-	-	-	A	9	-	/	A
15	0,00	0,00	-	0,00	0,00	-	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	-	A	17	-	/	A
18	0,00	0,00	-	0,00	0,00	-	-	-	A	18	-	/	A
24	0,00	49,00	+	0,00	0,00	-	-	-	A	24	-	/	A
3	23,00	65,00	+	22,00	66,00	+	+	+	P	3	+	+	P
5	23,00	66,00	+	23,00	65,00	+	+	+	P	5	+	+	P
8	22,00	66,00	+	22,00	66,00	+	+	+	P	8	+	+	P
13	24,00	65,00	+	24,00	65,00	+	+	+	P	13	+	+	P
16	24,00	66,00	+	24,00	65,00	+	+	+	P	16	+	+	P
20	25,00	66,00	+	24,00	66,00	+	+	+	P	20	+	+	P
22	0,00	0,00	-	0,00	0,00	-	-	-	A	22	+	+	P
23	23,00	66,00	+	23,00	65,00	+	+	+	P	23	+	+	P
1	17,00	65,00	+	17,00	66,00	+	+	+	P	1	+	+	P
6	17,00	66,00	+	18,00	65,00	+	+	+	P	6	+	+	P
10	18,00	65,00	+	17,00	65,00	+	+	+	P	10	+	+	P
11	17,00	65,00	+	17,00	65,00	+	+	+	P	11	+	+	P
12	17,00	65,00	+	17,00	65,00	+	+	+	P	12	+	+	P
14	18,00	66,00	+	18,00	65,00	+	+	+	P	14	+	+	P
19	18,00	65,00	+	18,00	65,00	+	+	+	P	19	+	+	P
21	19,00	66,00	+	19,00	65,00	+	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 24h

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	PA
13	PA
16	PA
20	PA
22	ND
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		C	Colonies aspect	Confir-mation
CP	MP	Result	CP	MP	Result							
2	0,00	0,00	-	0,00	0,00	-	-	A	2	-	/	A
4	0,00	54,05	+	0,00	0,00	!	-	A (FP)	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	A	7	-	/	A
9	11,20	0,00	-	0,00	0,00	-	-	A	9	-	/	A
15	0,00	0,00	-	0,00	0,00	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	A	17	-	/	A
18	9,61	0,00	-	0,00	0,00	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	A	24	-	/	A
3	25,56	64,93	+	0,00	0,00	!	+	P	3	+	+	P
5	24,97	64,93	+	0,00	0,00	!	+	P	5	+	+	P
8	25,23	65,42	+	25,60	64,83	+	+	P	8	+	+	P
13	27,12	64,87	+	32,17	64,08	+	+	P	13	+	+	P
16	25,29	65,40	+	0,00	0,00	!	+	P	16	+	+	P
20	24,79	64,85	+	25,60	64,33	+	+	P	20	+	+	P
22	23,93	64,86	+	25,29	64,34	+	+	P	22	+	+	P
23	26,93	65,00	+	26,53	64,55	+	+	P	23	+	+	P
1	19,32	64,84	+	18,73	64,71	+	+	P	1	+	+	P
6	19,52	65,00	+	19,12	64,60	+	+	P	6	+	+	P
10	18,17	64,69	+	18,20	64,59	+	+	P	10	+	+	P
11	19,25	64,89	+	20,78	64,38	+	+	P	11	+	+	P
12	19,47	64,86	+	22,55	64,01	+	+	P	12	+	+	P
14	20,12	64,91	+	22,74	64,15	+	+	P	14	+	+	P
19	18,68	64,79	+	18,46	64,28	+	+	P	19	+	+	P
21	18,84	64,77	+	19,87	64,29	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 22h

2	NA
4	NA (PP)
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	PA
13	PA
16	PA
20	PA
22	PA
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		D	Colonies aspect	Confir-mation
CP	MP	Result	CP	MP	Result							
2	0,00	0,00	-	0,00	0,00	-	-	A	2	-	/	A
4	0,00	50,71	+	0,00	0,00	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	A	7	-	/	A
9	0,00	0,00	-	0,00	0,00	-	-	A	9	-	/	A
15	0,00	50,36	+	0,00	0,00	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	A	17	-	/	A
18	0,00	48,80	+	0,00	0,00	-	-	A	18	-	/	A
24	0,00	49,85	+	0,00	0,00	-	-	A	24	-	/	A
3	19,65	65,98	+	19,54	65,92	+	+	P	3	+	+	P
5	18,09	65,92	+	17,88	65,93	+	+	P	5	+	+	P
8	18,88	66,15	+	18,43	66,15	+	+	P	8	+	+	P
13	17,73	65,93	+	17,48	65,94	+	+	P	13	+	+	P
16	20,82	65,92	+	20,63	65,90	+	+	P	16	+	+	P
20	19,05	65,95	+	18,63	66,09	+	+	P	20	+	+	P
22	21,50	66,03	+	21,28	66,10	+	+	P	22	+	+	P
23	19,13	65,79	+	18,63	65,92	+	+	P	23	+	+	P
1	16,77	66,17	+	16,98	65,61	+	+	P	1	+	+	P
6	16,87	65,92	+	16,57	66,08	+	+	P	6	+	+	P
10	16,76	65,70	+	16,75	65,51	+	+	P	10	+	+	P
11	16,75	65,81	+	16,77	65,87	+	+	P	11	+	+	P
12	16,89	65,92	+	16,96	64,94	+	+	P	12	+	+	P
14	16,74	65,87	+	16,54	65,96	+	+	P	14	+	+	P
19	16,82	66,03	+	16,51	66,12	+	+	P	19	+	+	P
21	17,05	66,03	+	16,62	66,03	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 22h

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	PA
13	PA
16	PA
20	PA
22	PA
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		E	Colonies aspect	Confir-mation
CP	MP	Result	CP	MP	Result							
2	0,00	50,33	+	0,00	0,00	-	-	A	2	-	/	A
4	0,00	0,00	-	0,00	0,00	-	-	A	4	-	/	A
7	0,00	0,00	!	0,00	0,00	-	-	A	7	-	/	A
9	0,00	0,00	-	0,00	0,00	-	-	A	9	-	/	A
15	0,00	0,00	-	0,00	0,00	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	A	17	-	/	A
18	0,00	66,05	+	0,00	0,00	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	A	24	-	/	A
3	19,44	65,11	+	18,58	64,94	+	+	P	3	+	+	P
5	19,77	64,96	+	18,87	64,86	+	+	P	5	+	+	P
8	19,56	65,09	+	18,69	65,01	+	+	P	8	+	+	P
13	19,46	64,66	+	18,32	64,62	+	+	P	13	+	+	P
16	19,53	64,98	+	18,55	64,89	+	+	P	16	+	+	P
20	19,07	64,81	+	18,43	64,77	+	+	P	20	+	+	P
22	19,15	64,84	+	18,50	64,83	+	+	P	22	+	+	P
23	19,04	64,77	+	18,49	64,98	+	+	P	23	+	+	P
1	19,26	65,24	+	18,06	65,06	+	+	P	1	+	+	P
6	18,80	65,08	+	18,06	64,96	+	+	P	6	+	+	P
10	15,93	64,72	+	18,06	64,68	+	+	P	10	+	+	P
11	18,83	64,72	+	17,78	64,72	+	+	P	11	+	+	P
12	18,79	64,60	+	17,74	64,64	+	+	P	12	+	+	P
14	18,87	64,72	+	17,62	64,63	+	+	P	14	+	+	P
19	18,73	64,90	+	17,90	64,86	+	+	P	19	+	+	P
21	18,56	64,70	+	17,96	64,79	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 24h

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	PA
13	PA
16	PA
20	PA
22	PA
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		F	Colonies aspect	Confir-mation
CP	MP	Result	CP	MP	Result							
2	0,00	0,00	-	0,00	0,00	-	-	A	2	-	/	A
4	0,00	65,50	+	0,00	0,00	-	-	A	4	-	/	A
7	0,00	49,22	+	0,00	0,00	-	-	A	7	-	/	A
9	0,00	66,09	+	0,00	0,00	-	-	A	9	-	/	A
15	0,00	0,00	-	27,54	64,60	+	-	A (FP)	15	-	/	A
17	0,00	65,38	+	0,00	65,73	+	+	P	17	-	/	A
18	0,00	65,92	+	0,00	65,01	+	+	P	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	A	24	-	/	A
3	21,66	65,11	+	17,72	64,73	+	+	P	3	+	+	P
5	23,79	64,40	+	18,67	64,59	+	+	P	5	+	+	P
8	24,62	64,96	+	19,56	64,60	+	+	P	8	+	+	P
13	23,78	64,39	+	20,88	62,99	+	+	P	13	+	+	P
16	25,38	64,44	+	21,71	63,31	+	+	P	16	+	+	P
20	0,00	0,00	-	17,91	64,51	+	+	P	20	+	+	P
22	21,30	64,82	+	17,30	64,61	+	+	P	22	+	+	P
23	0,00	0,00	-	0,00	0,00	-	-	A	23	-	/	A
1	19,06	65,05	+	17,72	65,06	+	+	P	1	+	+	P
6	20,17	64,80	+	17,89	64,45	+	+	P	6	+	+	P
10	21,00	63,25	+	17,60	64,41	+	+	P	10	+	+	P
11	19,78	64,63	+	17,64	64,51	+	+	P	11	+	+	P
12	19,67	64,61	+	17,66	64,46	+	+	P	12	+	+	P
14	20,01	64,66	+	17,78	64,45	+	+	P	14	+	+	P
19	20,31	64,81	+	17,29	64,66	+	+	P	19	+	+	P
21	22,07	64,60	+	17,24	64,51	+	+	P	21	+	+	P

Incubation time of the alternative method broth :

2	NA
4	NA
7	NA
9	NA
15	NA (PP)
17	PD
18	PD
24	NA
3	PA
5	PA
8	PA
13	PA
16	PA
20	PA
22	PA
23	NA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2						G	Colonies aspect	Confir-mation
CP	MP	Result	CP	MP	Result	Confirmation	Final result					
2	0,00	0,00	-	0,00	0,00	-	-	A	2	-	/	A
4	0,00	0,00	-	0,00	0,00	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	A	7	-	/	A
9	0,00	0,00	-	0,00	0,00	-	-	A	9	-	/	A
15	0,00	0,00	-	0,00	0,00	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	A	17	-	/	A
18	0,00	0,00	-	0,00	0,00	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	A	24	-	/	A
3	25,85	64,83	+	25,79	64,64	+	+	P	3	+	+	P
5	27,55	64,71	+	27,11	64,62	+	+	P	5	+	+	P
8	27,23	64,80	+	27,17	64,68	+	+	P	8	+	+	P
13	26,82	64,70	+	26,15	64,51	+	+	P	13	+	+	P
16	0,00	0,00	-	0,00	0,00	-	-	A	16	+	+	P
20	25,69	64,71	+	25,17	64,67	+	+	P	20	+	+	P
22	0,00	0,00	-	0,00	0,00	-	-	A	22	+	+	P
23	0,00	0,00	-	0,00	0,00	-	-	A	23	+	+	P
1	21,06	64,76	+	21,46	64,70	+	+	P	1	+	+	P
6	21,00	64,66	+	21,24	64,60	+	+	P	6	+	+	P
10	20,78	64,69	+	20,56	64,35	+	+	P	10	+	+	P
11	21,10	64,78	+	20,67	64,54	+	+	P	11	+	+	P
12	21,01	64,69	+	20,58	64,45	+	+	P	12	+	+	P
14	20,83	64,69	+	20,14	64,58	+	+	P	14	+	+	P
19	20,79	64,76	+	20,66	64,74	+	+	P	19	+	+	P
21	20,77	64,68	+	20,63	64,46	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 24h

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	PA
13	PA
16	ND
20	PA
22	ND
23	ND
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2						H	Colonies aspect	Confir-mation
CP	MP	Result	CP	MP	Result	Confirmation	Final result					
2	0,00	0,00	-	0,00	0,00	-	-	A	2	+	+	P
4	0,00	0,00	-	0,00	0,00	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	A	7	+	+	P
9	0,00	0,00	-	0,00	0,00	-	-	A	9	-	/	A
15	0,00	0,00	-	0,00	0,00	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	A	17	-	/	A
18	0,00	0,00	-	0,00	0,00	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	A	24	-	/	A
3	0,00	0,00	-	0,00	0,00	-	-	A	3	+	+	P
5	0,00	0,00	-	0,00	0,00	-	-	A	5	+	+	P
8	20,89	65,34	+	19,80	65,24	+	+	P	8	-	/	A
13	0,00	0,00	-	8,93	0,00	-	-	A	13	-	/	A
16	0,00	0,00	-	0,00	0,00	-	-	A	16	-	/	A
20	0,00	0,00	-	0,00	0,00	-	-	A	20	-	/	A
22	23,28	65,17	+	21,87	64,82	+	+	P	22	-	/	A
23	0,00	0,00	-	0,00	0,00	-	-	A	23	-	/	A
1	19,49	65,57	+	17,61	65,14	+	+	P	1	+	+	P
6	18,25	65,18	+	17,52	65,15	+	+	P	6	+	+	P
10	18,75	64,69	+	17,11	64,93	+	+	P	10	+	+	P
11	18,97	65,27	+	17,31	65,01	+	+	P	11	+	+	P
12	18,29	65,02	+	17,12	65,00	+	+	P	12	+	+	P
14	18,28	65,09	+	17,43	64,89	+	+	P	14	+	+	P
19	18,23	65,11	+	17,43	64,85	+	+	P	19	+	+	P
21	17,90	65,01	+	17,29	64,46	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 24h

2	ND
4	NA
7	ND
9	NA
15	NA
17	NA
18	NA
24	NA
3	ND
5	ND
8	PD
13	NA
16	NA
20	NA
22	PD
23	NA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		I	Colonies aspect	Confir-mation
CP	MP	Result	CP	MP	Result							
2	0,00	0,00	-	0,00	0,00	-	-	A	2	-	/	A
4	0,00	0,00	-	0,00	0,00	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	A	7	-	/	A
9	0,00	0,00	-	0,00	0,00	-	-	A	9	-	/	A
15	0,00	0,00	-	0,00	0,00	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	A	17	-	/	A
18	0,00	0,00	-	0,00	0,00	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	A	24	-	/	A
3	22,88	65,22	+	23,26	64,92	+	+	P	3	-	/	A
5	0,00	0,00	-	0,00	0,00	-	-	A	5	-	/	A
8	0,00	0,00	-	0,00	0,00	-	-	A	8	-	/	A
13	0,00	0,00	-	0,00	0,00	-	-	A	13	-	/	A
16	0,00	0,00	-	0,00	0,00	-	-	A	16	-	/	A
20	0,00	0,00	-	0,00	0,00	-	-	A	20	-	/	A
22	0,00	0,00	-	0,00	0,00	-	-	A	22	+	+	P
23	0,00	0,00	-	0,00	0,00	-	-	A	23	+	+	P
1	17,94	65,46	+	18,02	65,15	+	+	P	1	+	+	P
6	18,01	65,12	+	17,89	64,83	+	+	P	6	+	+	P
10	17,91	64,82	+	18,64	64,13	+	+	P	10	+	+	P
11	17,86	65,02	+	18,01	64,80	+	+	P	11	+	+	P
12	18,27	65,03	+	18,73	64,40	+	+	P	12	+	+	P
14	18,14	64,92	+	18,25	64,72	+	+	P	14	+	+	P
19	18,16	64,87	+	18,50	64,67	+	+	P	19	+	+	P
21	18,00	64,71	+	18,22	64,66	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 23h

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PD
5	NA
8	NA
13	NA
16	NA
20	NA
22	ND
23	ND
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		J	Colonies aspect	Confirmation
CP	MP	Result	CP	MP	Result							
2	0,00	0,00	-	0,00	0,00	-	-	A	2	-	/	A
4	0,00	0,00	-	0,00	0,00	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	A	7	-	/	A
9	0,00	0,00	-	0,00	0,00	-	-	A	9	-	/	A
15	0,00	0,00	-	0,00	0,00	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	A	17	-	/	A
18	0,00	0,00	-	0,00	0,00	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	A	24	-	/	A
3	23,48	64,90	+	22,97	64,87	+	+	P	3	-	/	A
5	0,00	0,00	-	0,00	0,00	-	-	A	5	-	/	A
8	22,04	65,50	+	21,45	65,05	+	+	P	8	-	/	A
13	0,00	0,00	-	0,00	0,00	-	-	A	13	+	+	P
16	22,52	65,06	+	21,87	64,87	+	+	P	16	+	+	P
20	0,00	0,00	-	0,00	0,00	-	-	A	20	+	+	P
22	23,57	64,91	+	22,90	64,70	+	+	P	22	+	+	P
23	0,00	0,00	-	0,00	0,00	-	-	A	23	-	/	A
1	20,31	65,16	+	20,10	64,61	+	+	P	1	+	+	P
6	20,20	65,15	+	19,61	64,90	+	+	P	6	+	+	P
10	20,26	64,85	+	19,84	64,65	+	+	P	10	+	+	P
11	20,17	64,82	+	19,85	64,23	+	+	P	11	+	+	P
12	20,25	64,66	+	19,54	64,72	+	+	P	12	+	+	P
14	20,01	64,88	+	19,10	64,63	+	+	P	14	+	+	P
19	21,64	52,55	+	19,56	64,85	+	+	P	19	+	+	P
21	21,47	52,36	+	19,84	64,34	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 22h

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PD
5	NA
8	PD
13	ND
16	PA
20	ND
22	PA
23	NA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

No

Code	Alternative method								Code	Méthode de Référence			
	GENE-UP L. monocytogenes									ISO 11290-1/A1			
	L	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation		Final result	L	Aspect colonies	Confirmation
CP		MP	Result	CP	MP	Result							
2	0,00	0,00	-	0,00	0,00	-	-	-	A	2	-	/	A
4	0,00	50,88	+	0,00	0,00	-	-	-	A	4	-	/	A
7	0,00	53,79	+	0,00	0,00	-	-	-	A	7	-	/	A
9	8,92	0,00	-	0,00	0,00	-	-	-	A	9	-	/	A
15	0,00	49,34	+	0,00	0,00	-	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	-	A	17	-	/	A
18	0,00	53,73	+	0,00	0,00	-	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	-	A	24	-	/	A
3	23,76	64,86	+	23,29	64,77	+	+	+	P	3	+	+	P
5	22,03	64,74	+	21,85	64,70	+	+	+	P	5	+	+	P
8	23,31	64,99	+	22,73	64,85	+	+	+	P	8	+	+	P
13	21,47	64,59	+	21,06	64,68	+	+	+	P	13	+	+	P
16	25,25	64,94	+	24,48	64,75	+	+	+	P	16	+	+	P
20	0,00	48,90	+	0,00	0,00	-	-	-	A	20	+	+	P
22	0,00	49,31	+	0,00	0,00	-	-	-	A	22	+	+	P
23	22,93	64,84	+	21,90	64,74	+	+	+	P	23	+	+	P
1	18,70	64,83	+	19,12	64,81	+	+	+	P	1	+	+	P
6	18,05	64,86	+	17,79	64,77	+	+	+	P	6	+	+	P
10	17,99	64,76	+	17,96	64,52	+	+	+	P	10	+	+	P
11	18,26	64,68	+	17,76	64,63	+	+	+	P	11	+	+	P
12	18,22	64,79	+	17,74	64,57	+	+	+	P	12	+	+	P
14	18,42	64,88	+	17,86	64,69	+	+	+	P	14	+	+	P
19	18,57	64,83	+	17,51	64,86	+	+	+	P	19	+	+	P
21	18,25	64,50	+	17,34	64,73	+	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 22h

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	PA
13	PA
16	PA
20	ND
22	ND
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence			
	GENE-UP L. monocytogenes									ISO 11290-1/A1			
	M	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation		Final result	M	Aspect colonies	Confirmation
CP		MP	Result	CP	MP	Result							
2	0,00	0,00	-	0,00	0,00	-	-	-	A	2	-	/	A
4	0,00	0,00	-	0,00	0,00	-	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	-	A	7	-	/	A
9	0,00	0,00	-	0,00	0,00	-	-	-	A	9	-	/	A
15	0,00	0,00	-	0,00	0,00	-	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	-	A	17	-	/	A
18	0,00	50,61	+	0,00	0,00	-	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	-	A	24	-	/	A
3	29,28	65,20	+	28,47	65,29	+	+	+	P	3	+	+	P
5	27,82	65,06	+	27,47	65,17	+	+	+	P	5	+	+	P
8	0,00	0,00	-	0,00	0,00	-	-	-	A	8	+	+	P
13	27,93	65,06	+	27,46	64,99	+	+	+	P	13	+	+	P
16	27,96	65,40	+	27,21	65,29	+	+	+	P	16	+	+	P
20	27,35	64,99	+	26,88	64,85	+	+	+	P	20	+	+	P
22	27,43	64,97	+	26,89	64,85	+	+	+	P	22	+	+	P
23	27,97	65,11	+	27,62	64,90	+	+	+	P	23	+	+	P
1	21,78	65,31	+	21,18	65,45	+	+	+	P	1	+	+	P
6	23,75	65,14	+	23,68	65,38	+	+	+	P	6	+	+	P
10	24,83	65,09	+	24,46	65,02	+	+	+	P	10	+	+	P
11	24,68	65,03	+	24,45	65,14	+	+	+	P	11	+	+	P
12	24,17	65,02	+	23,96	65,08	+	+	+	P	12	+	+	P
14	25,44	65,09	+	25,29	64,98	+	+	+	P	14	+	+	P
19	24,46	65,04	+	23,70	64,83	+	+	+	P	19	+	+	P
21	23,53	64,88	+	23,14	64,77	+	+	+	P	21	+	+	P

Incubation time of the alternative method broth : 24h

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	ND
13	PA
16	PA
20	PA
22	PA
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		N	Aspect colonies	Confirmation
CP	MP	Result	CP	MP	Result							
2	0,00	0,00	-	0,00	0,00	-	-	A	2	-	/	A
4	0,00	0,00	-	0,00	0,00	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	A	7	-	/	A
9	0,00	0,00	-	0,00	0,00	-	-	A	9	-	/	A
15	0,00	0,00	-	0,00	0,00	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	A	17	-	/	A
18	0,00	0,00	-	0,00	0,00	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	A	24	-	/	A
3	24,19	62,41	+	22,52	64,37	+	+	P	3	+	+	P
5	23,54	64,49	+	23,70	64,61	+	+	P	5	+	+	P
8	22,69	64,73	+	23,05	64,81	+	+	P	8	+	+	P
13	20,18	64,46	+	20,10	64,42	+	+	P	13	+	+	P
16	21,51	64,64	+	21,21	64,66	+	+	P	16	+	+	P
20	22,43	64,39	+	21,82	64,62	+	+	P	20	+	+	P
22	19,91	63,98	+	19,80	64,50	+	+	P	22	+	+	P
23	21,52	64,45	+	21,52	64,49	+	+	P	23	+	+	P
1	20,28	64,70	+	19,48	64,84	+	+	P	1	+	+	P
6	20,09	63,57	+	19,76	64,09	+	+	P	6	+	+	P
10	19,66	64,15	+	18,84	64,60	+	+	P	10	+	+	P
11	19,50	64,52	+	18,69	64,72	+	+	P	11	+	+	P
12	20,19	65,05	+	18,29	64,43	+	+	P	12	+	+	P
14	19,01	64,50	+	19,10	64,45	+	+	P	14	+	+	P
19	19,20	64,68	+	19,00	64,69	+	+	P	19	+	+	P
21	19,13	64,65	+	18,67	64,59	+	+	P	21	+	+	P

Incubation time of the alternative method broth :

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	PA
13	PA
16	PA
20	PA
22	PA
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

26h

Code	Alternative method								Code	Méthode de Référence		
	GENE-UP L. monocytogenes									ISO 11290-1/A1		
	GENE-UP Analysis 1			GENE-UP Analysis 2			Confirmation	Final result		O	Aspect colonies	Confirmation
CP	MP	Result	CP	MP	Result							
2	0,00	0,00	-	0,00	0,00	-	-	A	2	-	/	A
4	0,00	0,00	-	0,00	0,00	-	-	A	4	-	/	A
7	0,00	0,00	-	0,00	0,00	-	-	A	7	-	/	A
9	0,00	50,60	+	0,00	0,00	-	-	A	9	-	/	A
15	0,00	48,56	+	0,00	0,00	-	-	A	15	-	/	A
17	0,00	0,00	-	0,00	0,00	-	-	A	17	-	/	A
18	0,00	0,00	-	0,00	0,00	-	-	A	18	-	/	A
24	0,00	0,00	-	0,00	0,00	-	-	A	24	-	/	A
3	20,48	65,60	+	20,12	66,04	+	+	P	3	+	+	P
5	19,15	65,82	+	19,22	66,01	+	+	P	5	+	+	P
8	19,75	65,42	+	19,07	65,96	+	+	P	8	+	+	P
13	18,76	65,39	+	17,97	66,08	+	+	P	13	+	+	P
16	17,56	66,04	+	16,83	66,04	+	+	P	16	+	+	P
20	19,00	65,99	+	18,55	65,95	+	+	P	20	+	+	P
22	18,14	65,99	+	17,82	66,04	+	+	P	22	+	+	P
23	17,55	65,82	+	16,98	65,81	+	+	P	23	+	+	P
1	16,76	66,16	+	16,71	66,05	+	+	P	1	+	+	P
6	17,04	65,52	+	16,67	65,99	+	+	P	6	+	+	P
10	16,69	65,63	+	16,45	65,85	+	+	P	10	+	+	P
11	16,96	65,62	+	16,61	66,19	+	+	P	11	+	+	P
12	17,11	65,60	+	16,75	66,08	+	+	P	12	+	+	P
14	16,91	66,07	+	16,33	66,09	+	+	P	14	+	+	P
19	16,87	66,06	+	16,50	65,94	+	+	P	19	+	+	P
21	16,90	66,07	+	16,22	65,91	+	+	P	21	+	+	P

Incubation time of the alternative method broth :

2	NA
4	NA
7	NA
9	NA
15	NA
17	NA
18	NA
24	NA
3	PA
5	PA
8	PA
13	PA
16	PA
20	PA
22	PA
23	PA
1	PA
6	PA
10	PA
11	PA
12	PA
14	PA
19	PA
21	PA

22h

APPENDIX 9

**RESULTS OF THE EXPERT LABORATORY
INTERLABORATORY STUDY OF SEPTEMBER**

Code	Alternative method					Code	Reference method		
	GENE-UP <i>Listeria monocytogenes</i>						ISO 11290-1/A1		
ISHA	CP	MP	Result	Confirmation	Final result	ISHA	Colonies aspect	Confirmation	Final result
1	0,00	0,00	-	-	A	1	-	/	A
5	0,00	0,00	-	-	A	5	-	/	A
11	0,00	0,00	-	-	A	11	-	/	A
12	0,00	0,00	-	-	A	12	-	/	A
17	0,00	0,00	-	-	A	17	-	/	A
19	0,00	0,00	-	-	A	19	-	/	A
21	0,00	0,00	-	-	A	21	-	/	A
23	0,00	0,00	-	-	A	23	-	/	A
3	0,00	0,00	-	-	A	3	+	+	P
7	23,80	64,01	+	+	P	7	+	+	P
8	21,47	65,06	+	+	P	8	+	+	P
9	21,23	64,85	+	+	P	9	-	/	A
15	21,21	64,71	+	+	P	15	+	+	P
16	22,05	65,05	+	+	P	16	-	/	A
18	21,57	64,63	+	+	P	18	+	+	P
22	21,14	64,75	+	+	P	22	+	+	P
2	21,69	64,78	+	+	P	2	+	+	P
4	20,96	64,88	+	+	P	4	+	+	P
6	21,25	64,92	+	+	P	6	+	+	P
10	21,82	64,77	+	+	P	10	+	+	P
13	22,42	64,93	+	+	P	13	+	+	P
14	22,32	64,98	+	+	P	14	+	+	P
20	21,23	64,62	+	+	P	20	+	+	P
24	20,86	64,89	+	+	P	24	+	+	P

Incubation time of the alternative method broth : 22 h

1	NA
5	NA
11	NA
12	NA
17	NA
19	NA
21	NA
23	NA
3	ND
7	PA
8	PA
9	PD
15	PA
16	PD
18	PA
22	PA
2	PA
4	PA
6	PA
10	PA
13	PA
14	PA
20	PA
24	PA

APPENDIX 10

RESULTS OF THE COLLABORATORS
INTERLABORATORY STUDY OF SEPTEMBER

A=absence, P=presence, +=test positive, -=test negative, /=test not realized

Code		Alternative method GENE-UP <i>Listeria monocytogenes</i>				Code		Reference method ISO 11290-1/A1		
P	CP	MP	Result	Confirmation	Final result	P	Colonies aspect	Confirmation	Final result	
1	0,00	0,00	-	-	A	1	-	/	A	
5	0,00	0,00	-	-	A	5	-	/	A	
11	0,00	0,00	-	-	A	11	-	/	A	
12	0,00	0,00	-	-	A	12	-	/	A	
17	0,00	0,00	-	-	A	17	-	/	A	
19	0,00	0,00	-	-	A	19	-	/	A	
21	0,00	0,00	-	-	A	21	-	/	A	
23	0,00	0,00	-	-	A	23	-	/	A	
3	19,71	65,12	+	+	P	3	-	/	A	
7	0,00	0,00	-	-	A	7	+	+	P	
8	18,10	65,23	+	+	P	8	+	+	P	
9	17,92	64,82	+	+	P	9	-	/	A	
15	18,04	64,80	+	+	P	15	+	+	P	
16	17,93	64,16	+	+	P	16	+	+	P	
18	0,00	0,00	-	-	A	18	+	+	P	
22	0,00	0,00	-	-	A	22	+	+	P	
2	18,03	64,80	+	+	P	2	+	+	P	
4	18,01	64,85	+	+	P	4	+	+	P	
6	17,87	64,98	+	+	P	6	+	+	P	
10	17,97	64,65	+	+	P	10	+	+	P	
13	17,69	64,77	+	+	P	13	+	+	P	
14	17,61	64,82	+	+	P	14	+	+	P	
20	17,60	64,91	+	+	P	20	+	+	P	
24	17,47	65,14	+	+	P	24	+	+	P	

Incubation time of the alternative method broth : 28

1	NA
5	NA
11	NA
12	NA
17	NA
19	NA
21	NA
23	NA
3	PD
7	ND
8	PA
9	PD
15	PA
16	PA
18	ND
22	ND
2	PA
4	PA
6	PA
10	PA
13	PA
14	PA
20	PA
24	PA

Code		Alternative method GENE-UP <i>Listeria monocytogenes</i>				Code		Reference method ISO 11290-1/A1		
Q	CP	MP	Result	Confirmation	Final result	Q	Colonies aspect	Confirmation	Final result	
1	0,00	0,00	-	-	A	1	-	/	A	
5	20,66	64,89	+	-	A (FP)	5	-	/	A	
11	0,00	0,00	-	-	A	11	-	/	A	
12	0,00	0,00	-	-	A	12	-	/	A	
17	0,00	0,00	-	-	A	17	-	/	A	
19	0,00	0,00	-	-	A	19	-	/	A	
21	0,00	0,00	-	-	A	21	-	/	A	
23	0,00	0,00	-	-	A	23	-	/	A	
3	21,17	64,95	+	+	P	3	+	+	P	
7	24,46	64,88	+	+	P	7	-	/	A	
8	22,46	64,58	+	+	P	8	+	+	P	
9	22,14	64,53	+	+	P	9	+	+	P	
15	0,00	0,00	-	-	A	15	+	+	P	
16	22,73	64,98	+	+	P	16	-	/	A	
18	20,96	64,28	+	+	P	18	-	/	A	
22	22,82	64,79	+	+	P	22	+	+	P	
2	21,88	64,84	+	+	P	2	+	+	P	
4	21,83	64,93	+	+	P	4	+	+	P	
6	22,26	64,98	+	+	P	6	+	+	P	
10	21,25	64,38	+	+	P	10	+	+	P	
13	20,63	64,85	+	+	P	13	+	+	P	
14	22,08	64,98	+	+	P	14	+	+	P	
20	20,63	64,69	+	+	P	20	+	+	P	
24	20,20	65,07	+	+	P	24	+	+	P	

Incubation time of the alternative method broth : 24 h

1	NA
5	NA (PP)
11	NA
12	NA
17	NA
19	NA
21	NA
23	NA
3	PA
7	PD
8	PA
9	PA
15	ND
16	PD
18	PD
22	PA
2	PA
4	PA
6	PA
10	PA
13	PA
14	PA
20	PA
24	PA

Code	Alternative method					Code	Reference method		
	GENE-UP <i>Listeria monocytogenes</i>						ISO 11290-1/A1		
R	CP	MP	Result	Confirmation	Final result	R	Colonies aspect	Confirmation	Final result
1	0,00	0,00	-	-	A	1	-	/	A
5	0,00	0,00	-	-	A	5	-	/	A
11	0,00	0,00	-	-	A	11	-	/	A
12	0,00	0,00	-	-	A	12	-	/	A
17	0,00	0,00	-	-	A	17	-	/	A
19	0,00	0,00	-	-	A	19	-	/	A
21	0,00	0,00	-	-	A	21	-	/	A
23	0,00	0,00	-	-	A	23	-	/	A
3	0,00	0,00	-	+	A (FN)	3	+	+	P
7	24,61	66,19	+	+	P	7	+	+	P
8	0,00	0,00	-	+	A (FN)	8	+	+	P
9	22,75	66,26	+	+	P	9	+	+	P
15	0,00	0,00	-	+	A (FN)	15	+	+	P
16	0,00	0,00	-	+	A (FN)	16	+	+	P
18	25,28	66,06	+	+	P	18	+	+	P
22	24,00	66,23	+	+	P	22	-	/	A
2	21,53	66,32	+	+	P	2	+	+	P
4	23,75	66,20	+	+	P	4	+	+	P
6	21,42	66,27	+	+	P	6	+	+	P
10	25,48	65,90	+	+	P	10	+	+	P
13	21,55	65,80	+	+	P	13	+	+	P
14	26,49	66,13	+	+	P	14	+	+	P
20	22,28	65,99	+	+	P	20	+	+	P
24	21,89	66,44	+	+	P	24	+	+	P

Incubation time of the alternative method broth : 23 h

1	NA
5	NA
11	NA
12	NA
17	NA
19	NA
21	NA
23	NA
3	ND (FN)
7	PA
8	ND (FN)
9	PA
15	ND (FN)
16	ND (FN)
18	PA
22	PD
2	PA
4	PA
6	PA
10	PA
13	PA
14	PA
20	PA
24	PA

Code	Alternative method					Code	Reference method		
	GENE-UP <i>Listeria monocytogenes</i>						ISO 11290-1/A1		
S	CP	MP	Result	Confirmation	Final result	S	Colonies aspect	Confirmation	Final result
1	0,00	0,00	-	-	A	1	+	-	A
5	0,00	0,00	-	-	A	5	-	-	A
11	0,00	0,00	-	-	A	11	-	-	A
12	0,00	0,00	-	-	A	12	-	-	A
17	9,68	0,00	-	-	A	17	-	-	A
19	0,00	0,00	-	-	A	19	-	-	A
21	0,00	0,00	-	-	A	21	-	-	A
23	0,00	0,00	-	-	A	23	-	-	A
3	19,57	65,13	+	+	P	3	+	+	P
7	18,13	64,95	+	+	P	7	-	-	A
8	20,68	64,82	+	+	P	8	+	+	P
9	19,23	64,98	+	+	P	9	-	-	A
15	0,00	0,00	-	-	A	15	+	+	P
16	0,00	0,00	-	-	A	16	+	+	P
18	0,00	0,00	-	-	A	18	+	+	P
22	0,00	0,00	-	-	A	22	-	-	A
2	18,22	64,99	+	+	P	2	+	+	P
4	18,57	64,97	+	+	P	4	+	+	P
6	18,43	64,98	+	+	P	6	+	+	P
10	17,32	64,99	+	+	P	10	+	+	P
13	17,89	64,87	+	+	P	13	+	+	P
14	18,93	64,92	+	+	P	14	+	+	P
20	18,58	65,00	+	+	P	20	+	+	P
24	19,85	65,15	+	+	P	24	+	+	P

Incubation time of the alternative method broth : 24 h

1	NA
5	NA
11	NA
12	NA
17	NA
19	NA
21	NA
23	NA
3	PA
7	PD
8	PA
9	PD
15	ND
16	ND
18	ND
22	NA
2	PA
4	PA
6	PA
10	PA
13	PA
14	PA
20	PA
24	PA